

HLA-DRB and HLA-DQ genetic variability in patients with aspirin-exacerbated respiratory disease

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ABSTRACT

Background: Major histocompatibility complex (MHC) class II is involved in T-cell activation, cytokine secretion, and induction of immune responses. Cytokines, staphylococcus super antigens, and eosinophil activation are proposed to play important roles in aspirin-exacerbated respiratory disease (AERD).

Objectives: This study is aimed at investigating the association of HLA-DRB and DQ genetic variabilities in patients with AERD.

Methods: A genetic association analysis in three different groups, including 33 patients with AERD, 17 patients with aspirin-tolerant asthma (ATA), and 100 healthy controls was performed. Oral aspirin challenge (OAC) test was performed to identify aspirin hypersensitivity. Pulmonary function test (PFT) was performed for all patients. Eosinophil percentage in nasal smear and peripheral blood and serum immunoglobulin (Ig)E were investigated. HLA-DRB, HLA-DQA1, and HLA-DQB1 were genotyped using polymerase chain reaction.

Results: HLA-DQB1*0302 (OR, 5.49, 95% confidence interval [CI], (2.40–12.59)), HLA-DQA1*0301 (OR, 2.90, 95%CI, (1.49–5.67)), HLA-DRB4 (OR, 2.94, 95%CI, (1.61–5.36)), and HLA-DRB1*04 (OR, 3.19, 95%CI, (1.57–6.47)) were higher in patients with AERD compared with controls. In patients with AERD, HLA-DQB1*0301 (OR, 0.22, 95%CI, (0.09–0.54)), HLA-DQA1*0501 (OR, 0.42, 95%CI, (0.21–0.81)), HLA-DRB1*11 (OR, 0.30, 95%CI, (0.12–0.73)), and HLA-DRB3 (OR, 0.38, 95%CI, (0.21–0.70)) were significantly lower compared with healthy controls. Patients with AERD had lower frequencies of HLA-DQB1*0301 (OR, 0.27, 95%CI, (0.08–0.86)), and HLA-DRB1*011 (OR, 0.27, 95%CI, (0.08–0.86)) compared with ATA. Haplotypes of HLA-DRB1*04/DQA1*0301/DQB1*0302 (OR, 4.25, 95%CI, (1.94–9.29)) and HLA-DRB1*07/DQA1*0201/DQB1*0201 (OR, 3.52, 95%CI, (1.54–8.06)) were higher in patients with AERD compared with controls (all $p < 0.05$).

Conclusions: Results of this study suggest that HLA-DQB1*0302 and HLA-DRB1*04 and their related haplotypes are genes involved in predisposing patients to AERD, whereas HLA-DQB1*0301 and HLA-DRB1*011 have negative association with AERD.

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Aspirin is one of the most widely used drugs worldwide.¹ Intake of high doses of aspirin is associated with several side effects, including gastrointestinal ulcer, bleeding, and tinnitus.¹ Urticaria/angioedema and decline in lung function after ingestion of aspirin are seen in a group of patients that are regarded as aspirin hypersensitive.^{1,2} Aspirin hypersensitivity is uncommon in the general population with prevalence of 0.6%–2.5%.² Aspirin hypersensitivity is more prevalent among individuals with certain diseases.² Patients with asthma have a 10-fold increased risk of aspirin hypersensitivity compared with the general population²; up to 20% of patients with asthma might show aspirin-hypersensitivity phenotype. In patients with aspirin-intolerant asthma (AIA), ingestion of aspirin may lead to the acute asthma attack and the emergency department visit.¹ This phenomenon is not limited to aspirin and all of the nonsteroidal antiinflammatory drugs (NSAID) exert high percentage of cross sensitivity owing to inhibition of cyclooxygenase enzymes.² Aspirin and NSAID avoidance is usually recommended to patients. Aspirin desensitization will be offered to those with severe refractory asthma or sinusitis.¹

Chronic rhinosinusitis with nasal polypsis (CRSwNP), asthma, and aspirin hypersensitivity make up “Samter’s triad,” which is also called aspirin-exacerbated respiratory disease (AERD).² The pathogenesis of AERD is not fully understood. Modifications of eicosanoid metabolism and excess metabolism of arachidonic acid to cysteinyl-leukotrienes have been suggested as underlying mechanisms of AERD, justifying the utility of leukotriene inhibitors in the management of patients.^{1,3,4} Cysteinyl-leukotriene generation is also dependent on the platelet-adherent granulocytes and T-prostanoid receptors.³ Consistently, those carrying minor allele of rs6962291 in thromboxane A2 gene are protected against AIA possibly due to the lower catalytic activity of thromboxane A synthesis.⁴ In addition, eosinophil activation is a key feature of AIA and nasal polyp formation.^{5,6} Recently, Choi *et al.* showed an eosinophil activation phenotype parallel to increased expression of eosinophilic cationic protein during both early and late stages of responses of patients with AERD.⁶ Activation of the immune system and secretion of several cytokines have been linked to eosinophil differentiation and activation and promotion of inflammatory milieu in patients with CRSwNP⁷ and AERD.⁸

Major histocompatibility complex (MHC) class II antigens are present in cell surface of antigen presenting cells and B cells. MHC class II molecules bind to extracellular antigens and present them to the immune system. MHC proteins are involved in CD4⁺ T-cell interactions with other immune cells resulting in the production of several cytokines. Hence, MHC molecules are important players in the immune responses. HLA-DPB1*0301, member of MHC class II family, has been reported as a susceptibility allele to AIA in several populations.^{9–11} Interestingly, HLA-DPB1*0301 may predict leukotrien inhibitor requirement in patients with AERD.¹² By contrast, limited data exist on the association of other HLA genes with AERD. Accordingly, this study is aimed at investigating the association of HLA-DRB1, HLA-DQA1, and HLA-DQB1 with AERD.

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METHODS

Patients

A case control study was conducted on 50 adult patients with CRSwNP and asthma and 100 healthy controls. Patients with asthma and CRSwNP were further categorized into aspirin-tolerant asthma (ATA) and AERD with respect to oral aspirin challenge (OAC) test. Nasal polyps were diagnosed using endoscopic evaluation of the nasal cavity and scored as demonstrated in Supplementary file 1.¹³ Asthma was diagnosed based on the patient's symptoms and pulmonary function tests (PFTs). Dyspnea and wheezing during the past five years plus one of the after PFT results were regarded as asthma (using VmaxSeries 2130 AutoboxSpirometry; Sensor Medics, Yorba Linda, CA); 1) airway hyperreactivity to less than 10-mg/mL PC20 Methacholine; 2) airway reversibility with more than 15% increase in forced expiratory volume in the first second (FEV1) plus 200 mL after inhalation of a short-acting bronchodilator; or 3) more than 20% increase in FEV1 after two weeks of treatment with long-acting bronchodilators. Asthma staging was done in accordance with Global Initiative for Asthma guidelines (Table 1).¹⁴ Medical records of patients were reviewed for the age of disease onset, medications, and previous polyp surgeries. Patients were asked about history of aspirin or NSAID consumption and hypersensitivity to these kinds of drugs. A history of having each of 1) dyspnea, 2) wheezing, 3) nasal blockage, 4) skin eruption, or 5) loss of consciousness after ingestion of aspirin or NSAIDs was considered as positive history of aspirin intolerance. Healthy controls without any inflammatory, autoimmune, or allergic diseases as well as no records of cancer, chronic, or active infectious diseases were selected randomly from blood donors at an Iranian blood transfusion center. In addition, controls were also examined by one clinician to rule out nasal polyp, anosmia, rhinitis, and asthma. Cases and controls were from the same Persian ethnicity and unrelated. The local ethics committee approved the study protocol, and all of the participants signed the informed consents before enrollment.

Oral Aspirin Challenge

In the outpatient setting, one day OAC test was done for all patients without severe asthma.¹⁵ PFT was performed before OAC to measure

FEV1. Those with FEV1 more than 70% predicted were selected to be included in this study. PFTs were performed in accordance with the American Thoracic Society guidelines using VmaxAutobox Spirometry (Sensor Medics, Yorba Linda, CA). OAC was initiated with placebo capsules administered every 1.5 hours for a total of four administrations in the first day. In the next day, OAC was done with increasing doses of aspirin from 20, 40, 80, and 160 to the maximum dose of 320 mg administered every 90 minutes. However, intervals of aspirin administration were extended to three hours in those with a history of asthma exacerbation during the past six months, those with history of severe reaction to aspirin or NSAIDs. Rate (%) of decline in FEV1 was calculated as follows: prechallenge minus postchallenge FEV1 divided by prechallenge FEV1. One physician visited the patients hourly to record the positive reaction. More than 20% decrease in FEV1 or 15%–19% decrease in FEV1 accompanied by naso-ocular reactions (tearing, nasal congestion), urticaria, or angioedema were considered as positive reactions. Patients were kept in the clinic for at least three hours after completion of the test. Those with positive OAC were regarded as AIA, whereas those with negative results were considered as ATA. A participant with the classic triad of asthma, CRSwNP and aspirin intolerance were deemed AERD.

Eosinophil Percentage, Skin-Prick Test (SPT), and Serum Immunoglobulin (Ig)E

Eosinophil percentage was investigated on both of nasal smears and peripheral venous blood of the patients. Nasal secretions of the patients were collected using appropriate swab and stained by hematoxylin and eosin. A total of 10 high-power fields were reviewed by a blinded pathologist and mean of proportion of eosinophils to all infiltrated leukocytes were recorded. Peripheral blood samples were drawn in the morning before the application of OAC and SPT. An automated hematology analyzer (Sysmex XE-2100) was used to record eosinophil counts. Absolute number of eosinophils more than 0.5×10^9 cells/mL was considered significant.¹⁶ Total serum level of IgE was investigated by spectrophotometry.¹⁷ Standard extracts of 24 allergens were used to test the cutaneous hyperreactivity of the patients.¹⁸ Positive and negative controls of the procedure were histamine and normal saline deletions applied to the forearm of the

Table 1 Demographics of participants

Variables (%)	Asthma With CRSwNP (Number 50)	AERD (Number 33)	ATA (Number 17)	p Value
Age (years)	39.0 ± 11.2	39.3 ± 12.3	38.4 ± 9.1	0.787
Sex				
Female	32 (64%)	22 (66.6%)	10 (58.8%)	0.688
Male	18 (36%)	11 (33.4%)	7 (41.1%)	
Asthma				
Controlled	14 (28%)	11 (33.2%)	1 (17.7%)	0.364
Partly controlled	21 (42%)	11 (33.4%)	10 (58.8%)	
Uncontrolled	15 (30%)	11 (33.4%)	4 (23.5%)	
Onset of diseases				
Asthma	12 (24%)	5 (15.1)	7 (41.2%)	0.041#
Rhinosinusitis	38 (76%)	28 (84.9%)	10 (58.8%)	
Polyp score	4.1 ± 1.0	4.2 ± 0.9	4.0 ± 1.2	0.387
Previous polypectomy (yes)	37 (74%)	26 (78.7%)	11 (64.7%)	0.444
Base line FEV1 (predicted %)	90.1 ± 11.7	91.4 ± 13.0	87.6 ± 8.5	0.279
Serum IgE >100	33 (66%)	22 (66.6%)	11 (64.7%)	0.890
Nasal smear eosinophilia more than 10%	8 (16%)	5 (15.1%)	3 (17.6%)	0.820
Serum eosinophil counts >450	45 (90%)	29 (87.8%)	16 (94.1%)	0.486
Positive SPT	26 (52%)	18 (54.5%)	8 (47.0%)	0.616
Previous immunotherapy	7 (14%)	4 (12.1%)	3 (17.6%)	0.624
Positive history of aspirin intolerance	15 (30%)	12 (36.3%)	3 (17.6%)	0.151

p Values were calculated by comparing AIA with ATA using independent t-test (for continues variables) or χ^2 test (for categorical variables). p Values of less than 0.05 were considered significant. Onset of disease: Patient was recognized first due to lower respiratory tract symptoms (asthma) or upper respiratory tract symptoms (rhinosinusitis). Age is expressed in year.

patients. After 20 minutes, the results were deemed positive if the wheal area was greater than 8 mm³ compared with the negative control. SPT was done under supervision of a blinded physician blinded to the results of other investigations.

Genotyping

Genotyping was performed on 10 mL of venous blood. Samples were kept with ethylene-diamine-tetra-acetic acid at -80°C until investigation. DNA was extracted using salting out method. Genomic DNA was extracted from peripheral blood leukocytes by phenol-chloroform method. HLA DRB1, DQA1, and DQB1 typing was performed by polymerase chain reaction based on sequence specific primers (PCR-SSP), using Olerup and Zetterquist method.¹⁹ The HLA-DRB, DQA and DQB primers were kindly supplied by the collaborative transplant study (Heidelberg, Germany) as reported previously.^{20,21} In the standard kit, low resolution sequence specific primers, Ampli TAQ DNA polymerase was used from the Roche (Basel, Switzerland). The PCR reactions were carried out in 10-μL volumes. The samples were amplified in techne genius thermal cycles. Samples were amplified after initial denaturation at 94°C for two minutes, followed by 10 cycles of 94°C denaturation for 10 seconds, 65°C annealing and extension for 60 seconds, and finally 20 cycles of 94°C denaturation for 10 seconds, 61°C annealing for 50 seconds, and 72°C extension for 30 seconds. After amplification, the PCR product was electrophoresed on an agarose gel at 170 voltage for 15 minutes and then on a UV transilluminator, the gel was interpreted for specific bands.

The haplotypes were calculated according to the Iranian population specific linkage disequilibrium pattern among HLA-DRB, HLA-DQA, and HLA-DQB alleles.²² Frequency of each genotype and haplotypes were estimated and compared between patients and controls and those with and without positive OAC.

Statistical Analysis

Epi Info statistical software (version 6.2; World Health Organization, Geneva, Switzerland) was used to analyze the data. Categorical variables were expressed as frequencies and compared using χ^2 test and Fisher's exact test was performed where applicable. Genotype frequencies were determined by direct gene counting and odds ratio and 95% confidence interval (OR, 95%CI) were estimated to compare frequencies between patients and controls and those with and without positive OAC. Bonferroni corrections for the multiple comparisons. Continued variables were expressed as mean $\pm$ standard deviation and compared using independent Student's *t*-test. Probability less than 0.05 was considered statistically significant. All tests were two tailed.

RESULTS

Demographic Characteristics of Patients With AERD

Of 50 patients with asthma and CRSwNP, 33 (66%) had AIA and were categorized as AERD. Interestingly, inconsistency existed between self-reported aspirin intolerance and the real status confirmed by OAC test. Of those with ATA according to OAC test, 3 (17.6%) claimed having aspirin intolerance, whereas only 12 (36.3%) of patients with AERD were aware of their aspirin hypersensitivity. No significant differences were observed in terms of age, sex, atopy (positive SPT), increased IgE level (more than 100 IU/mL), and eosinophil percentage whether in nasal smear or peripheral blood, and severity of asthma between patients with AERD and ATA (Table 1). Mean age of participants with AERD at the time of enrollment was 39.3 $\pm$ 12.3 years versus 38.4 $\pm$ 9.1 years in those with ATA (*p* = 0.787). Mean age of disease onset (diagnosed by a physician) was 26.7 $\pm$ 10.3 years in patients with AERD and 27.4 $\pm$ 8.3 years in patients with ATA (*p* = 0.799).

HLA Genetic Variabilities

HLA-DRB, HLA-DQA1, and HLA-DQB1, all located on the short arm of chromosome 6, were genotyped in this study. Some HLA genetic markers were higher in patients with AERD compared with healthy controlled, conferring a possible genetic risk factor for AERD. HLA-DQB1*0302 (OR, 5.49, 95%CI (2.40–12.59)), HLA-DQA1*0301 (OR, 2.90, 95%CI (1.49–5.67)), HLA-DRB4 (OR, 2.94, 95%CI (1.61–5.36)), and HLA-DRB1*04 (OR, 3.19, 95%CI (1.57–6.47)) were higher in patients with AERD (all *p* < 0.05) (Table 2). Although none of these alleles were significantly higher in patients with ATA compared with controls, there was no significant difference between ATA and AERD in terms of these genetic markers.

In patients with AERD, HLA-DQB1*0301 (OR, 0.22, 95%CI (0.09–0.54)), HLA-DQA1*0501 (OR, 0.42, 95%CI (0.21–0.81)), HLA-DRB1*11 (OR, 0.30, 95%CI (0.12–0.73)), and HLA-DRB3 (OR, 0.38, 95%CI (0.21–0.70)) were significantly lower compared with healthy controls (all *p* < 0.05) (Table 2). Of possibly protective genetic markers of AERD, HLA-DQB1*0301 and HLA-DRB1*11 were not different between ATA and healthy controls, implying that they are more specifically linked to aspirin hypersensitivity compared with CRSwNP and asthma. Consistently, we found that patients with AERD had lower frequencies of HLA-DQB1*0301 (OR, 0.27, 95%CI (0.08–0.86)) and HLA-DRB1*11 (OR, 0.27, 95%CI (0.08–0.86)) compared with patients with asthma, CRSwNP, and aspirin tolerance (regarded as ATA). On the other hand, HLA-DRB3 had lower frequency in patients with AERD or ATA compared with healthy controls, and no significant difference was found between these two groups of AERD and ATA participants (OR, 0.84, 95%CI (0.34–2.06)).

Haplotype Analysis

Haplotypes of HLA-DRB1*04/ DQA1*0301/ DQB1*0302 (OR, 4.25, 95%CI (1.94–9.29)) and HLA-DRB1*07/ DQA1*0201/ DQB1*0201 (OR, 3.52, 95%CI (1.54–8.06)) were higher in patients with AERD compared with controls (all *p* < 0.05). Such an association was not found in patients with ATA; HLA-DRB1*04/ DQA1*0301/ DQB1*0302 (OR, 2.29, 95%CI (0.76–6.83)) and HLA-DRB1*07/ DQA1*0201/ DQB1*0201 (OR, 2.48, 95%CI (0.82–7.47)). On the other hand, HLA-DRB1*15/ DQA1*0103/ DQB1*0601 was only of higher percentage in patients with ATA in comparison with controls (OR, 3.35, 95%CI (1.16–9.66)). HLA-DRB1*11/ DQA1*0501/ DQB1*0301 was the only haplotype with significantly lower frequencies in patients with AERD compared with controls (OR, 0.30, 95%CI (0.12–0.73)). Table 3 summarizes haplotype frequencies in participants.

DISCUSSION

This study demonstrated that a significant HLA generic variability between patients with AERD compared with both healthy controls and those with asthma and CRSwNP exists but without aspirin hypersensitivity. Carriage of HLA-DRB1*11, HLA-DQB1*0301, HLA-DQA1*0501, and HLA-DRB3 was less frequent among patients with AERD. On the other hand, HLA-DQB1*0302, HLA-DQA1*0301, HLA-DRB4, and HLA-DRB1*04 were significantly more common among patients with AERD. These data shed light on a possible genetic predisposition to AERD involving HLA genes and suggest involvement of immune system in pathophysiology of AERD.

AERD is a severe chronic airway disease which consists of three major components of CRSwNP, asthma, and aspirin intolerance previously regarded as Samter's triad.² The pathology behind AERD is not well understood and therapeutic responses are poor. More severity of symptoms and more reductions of quality of life of afflicted patients is expected.² Severe polyp score is associated with an increased chance of aspirin intolerance in patients with CRSwNP and asthma, whereas atopy, blood and nasal smear eosinophilia, and asthma severity do not predict aspirin intolerance.²³ Despite poor insight into pathology of AERD, an interaction between genetic and

Table 2 Genotype analysis of asthma phenotype with CRSwNP versus healthy controls

Genotype	Healthy Controls	Asthma With CRSwNP	AERD	ATA	OR(95%CI) ¹	OR(95%CI) ²	OR(95%CI) ³	OR(95%CI) ⁴
N (%)	(Alleles, 200)	(Alleles, 100)	(Alleles, 66)	(Alleles, 34)				
HLA-DRB1*0101	11 (5.5%)	5 (5%)	3 (4.5%)	2 (5.8%)	0.90 (0.30–2.67)	0.81 (0.22–3.02)	1.07 (0.22–5.07)	0.76 (0.12–4.79)
HLA-DRB1*15	25 (12.5%)	12 (12%)	6 (9%)	6 (17.6%)	0.95 (0.45–1.98)	0.70 (0.27–1.78)	1.50 (0.56–3.98)	0.46 (0.13–1.57)
HLA-DRB1*16	13 (6.5%)	9 (9%)	9 (13.6%)	0	1.42 (0.58–3.45)	2.27 (0.92–5.58)	NA	NA
HLA-DRB1*0301	20 (10%)	7 (7%)	6 (9%)	1 (2.9%)	0.67 (0.27–1.66)	0.90 (0.34–2.34)	0.27 (0.03–2.10)	3.3 (0.38–28.59)
HLA-DRB1*04	21 (10.5%)	23 (23%)	18 (27.3%)	5 (14.7%)	2.54 (1.33–4.87)	3.19 (1.57–6.47)	1.46 (0.51–4.20)	2.17 (0.72–6.48)
HLA-DRB1*07	13 (6.5%)	13 (13%)	8 (12%)	5 (14.7%)	2.14 (0.95–4.83)	1.98 (0.78–5.02)	2.48 (0.82–7.47)	0.80 (0.24–2.66)
HLA-DRB1*08	3 (1.5%)	2 (2%)	1 (1.5%)	1 (2.9%)	1.34 (0.22–8.15)	1.01 (0.10–9.88)	1.98 (0.20–19.70)	0.50 (0.03–8.37)
HLA-DRB1*0901	6 (3%)	2 (2%)	2 (3%)	0	0.65 (0.13–3.32)	1.01 (0.19–5.13)	NA	NA
HLA-DRB1*1001	8 (4%)	1 (1%)	0	1 (2.9%)	0.24 (0.02–1.96)	NA	0.72 (0.08–6.00)	NA
HLA-DRB1*11	50 (25%)	15 (15%)	6 (9%)	9 (26.4%)	0.52 (0.28–0.99)†	0.30 (0.12–0.73)	1.08 (0.47–2.46)	0.27 (0.08–0.86)†
HLA-DRB1*12	3 (1.5%)	1 (1%)	1 (1.5%)	0	0.66 (0.06–6.45)	1.01 (0.10–9.88)	NA	NA
HLA-DRB1*1301	9 (4.5%)	3 (3%)	1 (1.5%)	2 (5.8%)	0.65 (0.17–2.48)	0.32 (0.04–2.62)	1.32 (0.27–6.42)	0.24 (0.02–2.81)
HLA-DRB1*1302	5 (2.5%)	3 (3%)	3 (4.5%)	0	1.20 (0.28–5.15)	1.85 (0.43–7.99)	NA	NA
HLA-DRB1*1305	4 (2%)	0	0	0	NA	NA	NA	NA
HLA-DRB1*14	11 (6.5%)	4 (4%)	2 (3%)	2 (5.8%)	0.71 (0.22–2.30)	0.53 (0.11–2.48)	1.07 (0.22–5.07)	0.50 (0.06–3.71)
HLA-DRB3	102 (56%)	30 (30%)	19 (28.7%)	11 (32.4%)	0.41 (0.24–0.68)¶	0.38 (0.21–0.70)¶	0.45 (0.21–0.99)†	0.84 (0.34–2.06)
HLA-DRB4	40 (20%)	37 (37%)	28 (42.4%)	9 (26.4%)	2.34 (1.37–4.00)¶	2.94 (1.61–5.36)¶	1.44 (0.62–3.32)	2.04 (0.82–5.05)
HLA-DRB5	38 (19%)	21 (21%)	15 (22.7%)	6 (17.6%)	1.13 (0.62–2.05)	1.25 (0.63–2.46)	0.91 (0.35–2.36)	1.37 (0.47–3.93)
HLA-DQA1*0101	12 (6%)	5 (5%)	3 (4.5%)	2 (5.8%)	0.82 (0.28–2.40)	0.74 (0.20–2.72)	0.97 (0.20–4.58)	0.76 (0.12–4.79)
HLA-DQA1*0102	28 (14%)	17 (17%)	14 (21.2%)	3 (8.8%)	1.25 (0.65–2.42)	1.65 (0.81–3.37)	0.59 (0.17–2.07)	2.78 (0.74–10.45)
HLA-DQA1*0103	21 (10.5%)	9 (9%)	4 (6%)	5 (14.7%)	0.84 (0.37–1.91)	0.54 (0.18–1.66)	1.46 (0.51–4.20)	0.37 (0.09–1.49)
HLA-DQA1*0104	19 (9.5%)	5 (5%)	2 (3%)	3 (8.8%)	0.50 (0.18–1.38)	0.29 (0.06–1.31)	0.92 (0.25–3.30)	0.32 (0.05–2.03)
HLA-DQA1*0201	15 (7.5%)	13 (13%)	8 (12%)	5 (14.7%)	1.84 (0.84–4.04)	1.70 (0.68–4.21)	2.12 (0.71–6.29)	0.80 (0.24–2.66)
HLA-DQA1*0301	26 (13%)	25 (25%)	20 (30%)	5 (14.7%)	2.23 (1.20–4.11)†	2.90 (1.49–5.67)¶	1.15 (0.41–3.24)	2.52 (0.85–7.45)
HLA-DQA1*0401	1 (0.5%)	2 (2%)	1 (1.5%)	1 (2.9%)	4.06 (0.36–45.33)	3.06 (0.18–49.64)	6.03 (0.36–98.78)	0.50 (0.03–8.37)
HLA-DQA1*0501	78 (39%)	24 (24%)	14 (21.2%)	10 (29%)	0.49 (0.28–0.84)†	0.42 (0.21–0.81)¶	0.65 (0.29–1.43)	0.64 (0.25–1.66)
HLA-DQB1*0201	38 (19%)	20 (20%)	14 (21.2%)	6 (5.8%)	1.06 (0.58–1.94)	1.14 (0.57–2.28)	0.91 (0.35–2.36)	1.25 (0.43–3.63)
HLA-DQB1*0301	62 (31%)	15 (15%)	6 (9%)	9 (26.4%)	0.39 (0.21–0.73)¶	0.22 (0.09–0.54)¶	0.80 (0.35–1.81)	0.27 (0.08–0.86)†
HLA-DQB1*0302	11 (5.5%)	21 (21%)	16 (24.2%)	5 (14.7%)	4.56 (2.10–9.91)¶	5.49 (2.40–12.59)¶	2.96 (0.95–9.14)	1.85 (0.61–5.59)
HLA-DQB1*0303	8 (4%)	3 (3%)	3 (4.5%)	0	0.74 (0.19–2.86)	1.14 (0.29–4.43)	NA	NA
HLA-DQB1*0305	0	2 (2%)	2 (3%)	0	NA	NA	NA	NA
HLA-DQB1*0401	2 (1%)	2 (2%)	1 (1.5%)	1 (2.9%)	2.02 (0.28–14.55)	1.52 (0.13–17.07)	3.0 (0.26–34.02)	0.50 (0.03–8.37)
HLA-DQB1*0501	44 (22%)	20 (20%)	14 (21.2%)	6 (17.6%)	0.88 (0.48–1.60)	0.95 (0.48–1.88)	0.75 (0.29–1.95)	1.25 (0.43–3.63)
HLA-DQB1*0601	14 (7%)	9 (9%)	4 (6%)	5 (14.7%)	1.31 (0.54–3.14)	0.85 (0.27–2.70)	2.29 (0.76–6.83)	0.37 (0.09–1.49)
HLA-DQB1*0602	17 (8.5%)	4 (4%)	4 (6%)	0	0.44 (0.14–1.37)	0.69 (0.22–2.14)	NA	NA
HLA-DQB1*0604	4 (2%)	4 (4%)	2 (3%)	2 (5.8%)	2.04 (0.49–8.33)	1.53 (0.27–8.55)	3.06 (0.53–17.41)	0.50 (0.06–3.71)

NA = not applicable; CRSwNP = chronic rhinosinusitis; AERD = aspirin exacerbated airway diseases; ATA = aspirin tolerant asthma; OR(95%) = odds ratio and 95% confidence interval.

¹Frequency of HLA alleles compared between (asthma and CRSwNP) and healthy controls (as reference).

²Frequency of HLA alleles compared between AERD and healthy controls (as reference).

³Frequency of HLA alleles compared between ATA and healthy controls (as reference).

⁴Frequency of HLA alleles compared between AERD and ATA (as reference).

†, 0.01 < two sided *p* < 0.05.

¶, Two sided *P* < 0.01.

environment seems to exist.² HLA genes are located on the short arm of chromosome 6 near genes encoding tumor necrosis factor- α and some complement components. HLA allele and haplotypes have been linked to CRS,²⁴ asthma,^{25,26} aspirin hypersensitivity,¹¹ and AERD.²⁷ In patients with CRSwNP, genome-wide studies identified tumor necrosis factor- α polymorphisms as a genetic predisposition locus. In addition, the short arm of chromosome 6, which includes genes of MHCs, has been proposed as a genetic predisposition factor for the formation of chronic airway inflammation and nasal polyposis.²⁴ A large-scale genome-wide study on 10365 patients with asthma demonstrated that HLA-DQ genetic variabilities (rs9273349 on chromosome 6) were strongly associated with asthma both in adult and childhood onset asthma. Furthermore, HLA-DR was associated with the total serum IgE levels in asthmatic patients.²⁵ Interestingly, *HLA-DPB1*0301* has been associated with AERD,²⁷ and it is also proposed as a genetic marker of aspirin hypersensitivity.¹¹ Interestingly, *HLA-DPB1*0301* was strongly associated with the percentage of decline in

FEV1 after aspirin challenge test.²⁷ *HLA-DPB1*0301* polymorphisms are associated with tumor necrosis factor- α polymorphisms, which highlights genetic interaction of genes located on chromosome 6 in susceptibility to AIA.²⁸

Dekker *et al.* were the first to show strong association of HLA family genetic variabilities with AERD.¹⁰ *HLA-DRB1* and *HLA-DPB1* were genotyped using a restriction enzyme analysis and amplification of target regions by polymerase chain reaction in 59 patients with AIA, 57 ATA, and 48 healthy controls from England.¹⁰ They showed that *HLA-DPB1*0301* had higher frequency in patients with AIA compared with both healthy controls and ATA (19.5% versus 5.2% and 4.4%, OR = 4.4, 95%CI (1.6–12.1), and OR = 5.3, 95%CI (1.9–14.4), respectively).¹⁰ Further studies in the Korean population confirmed their findings only with a slight difference in frequency of *HLA-DPB1*0301*, most probably owing to different methods of genotyping, different methods of patient selection and aspirin challenge test, and finally racial differences.^{9,28,29} Although *HLA-DPB1*0301* represents a

Table 3 Haplotype analysis of asthma phenotype with CRSwNP versus healthy controls

Haplotypes	Healthy Controls	Asthma With CRSwNP	AERD	ATA	OR(95%CI) ¹	OR(95%CI) ²	OR(95%CI) ³	OR(95%CI) ⁴
DRB1/DQA1/DQB1	(200 alleles)	(100 alleles)	(66 alleles)	(34 alleles)				
DRB1*0101/DQA1*0101/ DQB1*0501	11 (5.5%)	5 (5%)	3 (4.5%)	2 (5.8%)	0.90 (0.30–2.67)	0.81 (0.22–3.02)	1.07 (0.22–5.07)	0.76 (0.12–4.79)
DRB1*0301/ DQA1*0501/DQB1*0201	20 (10%)	7 (7%)	6 (9%)	1 (2.9%)	0.67 (0.27–1.66)	0.90 (0.34–2.34)	0.27 (0.03–2.10)	3.30 (0.38–28.59)
DRB1*04/ DQA1*0301/ DQB1*0302	14 (7%)	21 (21%)	16 (24.2%)	5 (14.7%)	3.53 (1.70–7.29) #	4.25 (1.94–9.29) #	2.29 (0.76–6.83)	1.85 (0.61–5.59)
DRB1*04/ DQA1*0301/ DQB1*0305	2 (1%)	1 (1%)	1 (1.5%)	0	1.00 (0.08–11.16)	1.52 (0.13–17.07)	NA	NA
DRB1*04/ DQA1*0301/ DQB1*0201	5 (2.5%)	1 (1%)	1 (1.5%)	0	0.39 (0.04–3.41)	0.60 (0.06–5.23)	NA	NA
DRB1*07/ DQA1*0201/ DQB1*0201	13 (6.5%)	13 (13%)	8 (12%)	5 (14.7%)	2.14 (0.95–4.83)	3.52 (1.54–8.06)	2.48 (0.82–7.47)	0.80 (0.24–2.66)
DRB1*0801/ DQA1*0501/ DQB1*0301	3 (1.5%)	1 (1%)	1 (1.5%)	0	0.66 (0.06–6.45)	1.01 (0.10–9.88)	NA	NA
DRB1*0901/ DQA1*0301/ DQB1*0303	6 (3%)	2 (2%)	2 (3%)	0	0.65 (0.13–3.32)	1.01 (0.19–5.13)	NA	NA
DRB1*1001/ DQA1*0104/ DQB1*0501	8 (4%)	1 (1%)	0	1 (2.9)	0.24 (0.02–1.96)	NA	0.72 (0.08–6.00)	NA
DRB1*11/ DQA1*0501/ DQB1*0301	50 (25%)	14 (14%)	6 (9%)	8 (23.5%)	0.48 (0.25–0.93)	0.30 (0.12–0.73) #	0.92 (0.39–2.16)	0.32 (0.10–1.03)
DRB1*12/ DQA1*0501/ DQB1*0301	3 (1.5%)	1 (1%)	1 (1.5%)	0	0.66 (0.06–6.45)	1.01 (0.10–9.88)	NA	NA
DRB1*1301/ DQA1*0103/ DQB1*0603	9 (4.5%)	1 (1%)	1 (1.5%)	0	0.21 (0.02–1.71)	0.32 (0.04–2.62)	NA	NA
DRB1*1301/ DQA1*0501/ DQB1*0603	4 (2%)	3 (3%)	3 (4.5%)	0	1.51 (0.33–6.90)	2.33 (0.50–10.70)	NA	NA
DRB1*1302/ DQA1*0102/ DQB1*0602	1 (0.5%)	0	0	0	NA	NA	NA	NA
DRB1*1302/ DQA1*0102/ DQB1*0604	3 (1.5%)	2 (2%)	0	2 (5.8%)	1.34 (0.22–8.15)	NA	4.10 (0.65–25.52)	NA
DRB1*1401/ DQA1*0104/ DQB1*0501	11 (5.5%)	4 (4%)	2 (3%)	2 (5.8%)	0.71 (0.22–2.30)	0.53 (0.11–2.48)	1.07 (0.22–5.07)	0.50 (0.06–3.71)
DRB1*1404/ DQA1*0104/ DQB1*0501	2 (1%)	0	0	0	NA	NA	NA	NA
DRB1*15/ DQA1*0102/ DQB1*0501	2 (1%)	1 (1%)	0	1 (2.9%)	1.00 (0.08–11.16)	NA	3.00 (0.26–34.02)	NA
DRB1*15/ DQA1*0102/ DQB1*0601	2 (1%)	1 (1%)	1 (1.5%)	0	1.00 (0.08–11.16)	1.52 (0.13–17.07)	NA	NA
DRB1*15/ DQA1*0102/ DQB1*0602	8 (4%)	1 (1%)	1 (1.5%)	0	0.24 (0.02–1.96)	0.36 (0.04–3.00)	NA	NA
DRB1*15/ DQA1*0103/ DQB1*0601	12 (6%)	10 (10%)	4 (6%)	6 (17.6%)	1.74 (0.72–4.18)	1.01 (0.31–3.24)	3.35 (1.16–9.66) #	0.30 (0.07–1.15)
DRB1*16/ DQA1*0102/ DQB1*0501	13 (6.5%)	10 (10%)	9 (13.5%)	1 (2.9%)	1.59 (0.67–3.78)	2.27 (0.92–5.58)	0.43 (0.05–3.44)	5.21 (0.63–42.97)

NA = Not applicable; CRSwNP = chronic rhinosinusitis with nasal polyposis; AERD = aspirin-exacerbated airway diseases; ATA = aspirin-tolerant asthma; OR(95%CI) = odds ratio and 95% confidence interval.

¹Frequency of HLA alleles compared between (asthma and CRSwNP) and healthy controls (as reference).

²Frequency of HLA alleles compared between AERD and healthy controls (as reference).

³Frequency of HLA alleles compared between ATA and healthy controls (as reference).

⁴Frequency of HLA alleles compared between AERD and ATA (as reference)

0.01 < two sided $p < 0.05$.

¶ Two sided $P < 0.01$.

well-studied genetic marker of AIA and AERD, other members of HLA family were less studied in patients with either ATA or AERD. Recent genome-wide studies in patients with asthma introduced HLA-DQA1 and HLA-DQB1 as susceptibility loci.^{26,30} These loci were also linked to nasal polyposis in patients with CRS in several populations from Hungary,³¹ Mexico,³² and Turkey.³³ Furthermore, these studies demonstrated that genes encoding HLA-DR antigens might be associated with risk of nasal polyposis whether in normal population or patients with asthma.^{31–33} Accordingly, *HLA-DR* and *HLA-DQ* genes might be susceptibility loci of AERD which consists of CRSwNP, asthma, and aspirin intolerance.

Ramírez-Anguiano *et al.* found higher frequency of *HLA-DRB1*04* in patients with nasal polyposis (OR = 2.2, 95% CI, (1.2–4.2), *p* = 0.009).³² Similarly, Keles *et al.* reported higher frequency of *HLA-DRB1*04* in patients with AERD compared with healthy controls and patients with nasal polyposis or asthma.³³ In line with these findings, we found that *HLA-DRB1*04* was higher in patients with AERD; 27.3% of patients with AERD versus 10.5% of healthy controls were positive for *HLA-DRB1*04* (OR = 3.19 and 95%CI (1.57–6.47) (Table 3). Previously, *HLA-DQA1*0201* was found to be significantly higher in patients with AERD,³¹ whereas the present study failed to replicate such an association. Because the number of patients in two studies is similar, racial differences as well as technical differences in genotyping of HLA but not low power of present study might underlie these different results. In our study, *HLA-DQA1*0301*, *HLA-DQB1*0302*, and *HLA-DRB1*04* were associated with increased odds of AERD. These new findings strengthen the association of immune system as well as genetic background with development of AERD.

One of the strengths of this study was the presence of a group of patients with asthma, CRSwNP which were aspirin tolerant and were regarded as ATA. Genetic comparison of ATA with AERD revealed that *HLA-DRB1*11* and *HLA-DQB1*301* were less frequent among AERD, implying that these alleles are protective against aspirin sensitivity rather than asthma or CRSwNP. Less observation of *HLA-DRB1*11* and *HLA-DQB1*301* among patients with asthma and CRSwNP might originate from high percentage of AERD in this group of patients (66%). These HLAs could be regarded as genetic markers helping researchers identify aspirin-hypersensitivity phenotypes among patients with asthma and CRSwNP. Previously, Lee *et al.* proposed *HLA-DPB1*0301* as a genetic marker for AIA and *HLA-DRB1*1302-DQB1*0609* for AIU in Korean population.¹¹ They found that 23.4% of patients with AIA had *HLA-DPB1*0301*, whereas only 5.3% of healthy controls carried this allele.¹¹ In the present study, *HLA-DRB1*11* was found in 25% and 26.4% of healthy controls and patients with ATA, respectively. Similarly, *HLA-DQB1*301* was found in 31% and 26.4% of healthy controls and patients with ATA, respectively. However, Both *HLA-DRB1*11* and *DQB1*301* were lower than 10% in patients with AERD. Hence, a genetic signature comprised of absence of several alleles (for instance *HLA-DRB1*11* and *HLA-DQB1*1*) as well as presence of other predisposing alleles (for instance *HLA-DPB1*0301*) could provide an easy way to identify AERD using HLA typing of blood leukocytes derived from the venous blood. It is of importance with respect to expenses, dangers, and side effects associated with aspirin bronchoprovocation and OAC tests. Of note, aspirin challenge tests are time consuming and require close supervision of a healthcare provider. In the future, diagnostic and prognostic studies investigating the value of genetic signatures with aspirin challenge tests will pave the road to this approach to patient categorization. Recently, Shin *et al.*³⁴ used a set of only eight single nucleotide polymorphisms to discriminate AERD from ATA with promising results. In their experience, genetic signature was associated with 78% sensitivity and 88% specificity to identify AERD from other patients with asthma (area under curve = 0.9).³⁴ Future studies regarding the role of genetic signatures in diagnosis and management (pharmacokinetics) of patients with AERD are warranted.

With respect to the design of the present study, we were unable to draw a causality link between HLA gene variabilities and AERD.

However, patients usually carry these gene sequences from birth and de novo alterations in genetic material are far less possible. Accordingly, HLA gene variabilities may be regarded as the genetic etiology of AERD. The best study that could accept or reject this claim would be a birth cohort study of neonates genotyped for known susceptibility genes, including the HLA genes on chromosome 6. However, the method of genotyping should also be improved to reduce the required volume. Of more importance might be the investigation of therapeutic response of patients carrying special HLAs. It might help physicians to choose more efficient therapies with respect to patient's characteristics as considered in individualized based medicine.

In conclusion, this study showed significant *HLA-DR* and *HLA-DQ* genetic differences between patients with AERD and ATA or healthy controls exists. The role of the immune system in the development of AERD is highlighted.^{35,36} In addition, these data might provide hope for introducing genetic signatures in the diagnosis of aspirin hypersensitivity and AERD.

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