

Allergic and Nonallergic Asthma Have Distinct Phenotypic and Genotypic Features

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Keywords

Asthma · Human leukocyte antigens · Allergic asthma · Nonatopic

Abstract

Background: Identification of asthma phenotypes enables a better understanding and management of this heterogeneous disease. Studies have reported associations between human leukocyte antigens (HLA) and asthma in different populations, but the results have been inconclusive and they have rarely considered the distinct disease phenotypes. Our objective was to characterize allergic and nonallergic asthma phenotypes and evaluate possible associations with the HLA system. **Methods:** A total of 109 patients with asthma were prospectively followed during 2 years. They were divided into 2 groups, i.e., allergic and nonallergic asthma, according to their clinical history and skin prick test and serum-specific immunoglobulin E (IgE) results. The control group comprised 297 deceased donors of solid organs. Patients' features and HLA class I and II genotypes were assessed and compared. **Results:** This study showed different features between asthma phenotypes. Nonallergic patients were older at the onset of asthma symptoms and had a higher rate of intolerance to nonsteroidal anti-

inflammatory drugs. Allergic patients had higher total serum IgE levels, reported atopic dermatitis and rhinoconjunctivitis more frequently, and, unexpectedly, had a greater disease severity. New associations between the HLA genotypes and allergic and nonallergic asthma were identified. The HLA-B*42, HLA-C*17, HLA-DPA1*03, and HLA-DPB1*105 genotypes were associated with allergic asthma and the HLA-B*48 genotype with the nonallergic phenotype. The presence of the haplotype HLA-DPA1*03 DQA*05 was associated with allergic asthma, and the presence of HLA-DPA1*03 and the absence of HLA-DQA*05 with nonallergic asthma. **Conclusions:** Allergic and nonallergic asthma have distinct phenotypic and genotypic features. New associations between asthma phenotypes and HLA class I and II were identified.

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Introduction

Asthma is considered a syndrome or a disease with several phenotypes [1–5]. Common clinical conditions of this syndrome include similar respiratory symptoms, variable bronchial obstructions, bronchial hyperresponsiveness, and chronic inflammation with airway remodel-

ing. Not all patients have the same clinical outcome or adapt completely to the consensus approach, and therefore patients should be individually treated [1–5].

Various parameters have been used to define asthma phenotypes. The two main phenotypes with clear and distinct features are allergic and nonallergic asthma [6–7]. Approximately 90% of asthma cases in childhood and more than 50% of asthma cases in adults are allergic [8]. Allergic asthma tends to have an early onset and the attacks usually begin before the age of 12 years [2, 9]. It has a more benign course, a lower rate of intolerance to nonsteroidal anti-inflammatory drugs (NSAID), and an association with a personal and family history of other atopic diseases [2, 5]. Exacerbations of allergic asthma are typically associated with allergen exposure and patients have increased serum total immunoglobulin E (IgE) levels and specific IgE antibodies [10].

In contrast, nonallergic asthma has been described as a late-onset disease, with symptoms that often begin at 40 years of age or later [2, 8]. It occurs predominantly in females and it is more frequently associated with nonsteroidal anti-inflammatory hypersensitivity [11, 12]. A personal or family history of atopy is uncommon, and some studies have suggested that the lung function declines earlier in this phenotype than in the allergic asthma phenotype [2, 3, 5, 13, 14]. Eosinophilic airway inflammation is usually observed in both phenotypes; however, neutrophil infiltration is more common in nonallergic asthma [15].

Unlike allergic asthma, which has a well-characterized pathophysiology, the etiology and mechanisms involved in nonallergic asthma have not been elucidated. The disease could be triggered by one or more of the following mechanisms: hypersensitivity reactions to unidentified allergens such as fungi, recurrent or persistent infections, and autoimmune mechanisms [16–20].

The classification of asthma into allergic or nonallergic phenotypes is not merely conceptual; it has implications for the prognosis and treatment of the disease. For example, in cases of allergic asthma, environmental control is performed differently, and patients can benefit from anti-IgE and immunotherapy [21, 22].

Several studies focusing on asthma have been conducted to elucidate its pathophysiology, identify risk groups, and develop new treatments. The human leukocyte antigen (HLA) system has been indicated as a genetic factor that may be associated with the disease. The first studies described in the literature that addressed the association between asthma and the HLA system were performed with HLA class I [23–27] and subsequently with HLA

class II [28, 29] genes. Different criteria were used to characterize the patients in the studied populations, and their asthma phenotypes were not well established.

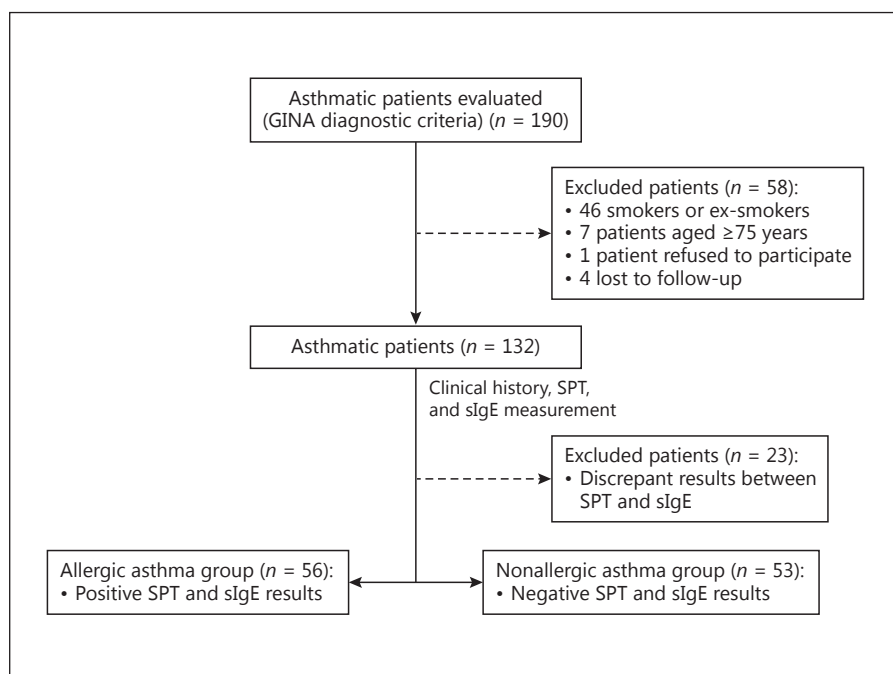
In 1971, a nonstatistically significant increase in the frequency of the HLA A1-B8 haplotype was reported among children with nonallergic asthma [23]. The same haplotype was also associated with asthmatic children with an IgA deficiency [24] and nasal polyposis [30]. An increased frequency of Bw6 among patients with nonallergic asthma was also reported, suggesting an association with a recessive inheritance model. However, the small number of participants and the evaluation of the inheritance pattern without a family study were questionable [26]. In contrast, a study performed in Chinese patients in Manchuria observed that HLA Bw61 was significantly associated with allergic asthma [31].

Another Chinese study assessing children who presented increased serum IgE levels and sensitivity to house dust mites on immediate-reading skin tests showed a positive and statistically significant association with HLA-DR2 and a negative association with HLA-DR4 [28]. HLA-DRB1*04 and HLA-DQA1*0301 antigen levels were significantly higher in Greek children who were sensitized to dust mites [32]. A study conducted in 2010 reported that the progression of asthma from initial symptoms to disease was slower among children carrying the HLA-DRB1*11 and DQB1*0301 antigens [33]. These antigens were reported as susceptibility markers for the development of allergic asthma in a Venezuelan population [34]. The HLA-DRB1*11 antigen presented a lower frequency among nonallergic patients [35]. In addition, a Chinese study suggested that the HLA-DQB1*0301 antigen has a protective effect on the development of asthma [36].

Aspirin-intolerant asthma, also known as aspirin-exacerbated respiratory disease, is considered a TH2-high phenotype of asthma. It has been recently shown that HLA-DQB1*0302 and HLA-DRB1*04 are associated with this distinct phenotype [37].

As described above, because of the pivotal role of HLA-II molecules in the presentation of allergen-derived peptides, HLA alleles have been extensively studied in hypothesis-driven candidate gene association studies for allergic sensitization and asthma [38]. However, only a few studies have evaluated the association between HLA and nonallergic asthma, the pathogenesis of which has not been elucidated. Nonallergic asthma could involve autoimmune mechanisms or even chronic nondiagnosed infections, and HLA alleles could play a role in this particular phenotype.

Fig. 1. Flow chart of the asthmatic patients included in this study and the final classification into allergic and nonallergic asthmatic groups. We considered allergic patients with a clinical history of asthma and rhinitis attacks triggered by aeroallergens, positive skin prick test (SPT; the mean wheal diameter induced by the allergen was 3 mm larger than the negative control wheal) and positive serum-specific immunoglobulin E (sIgE; greater than or equal to 0.35 kU/L). GINA, Global Initiative for Asthma.



The objective of this study was to characterize allergic and nonallergic asthma phenotypes and evaluate possible associations between HLA (class I and II) and these phenotypes.

Materials and Methods

This study was conducted in a prospective way in a cohort of asthmatic adults from the outpatient facility of the Clinical Immunology and Allergy Division, University of São Paulo, Brazil. The study protocol was approved by the Ethics Committee for Analysis of Research Projects of the Hospital das Clínicas da FMUSP (protocol No. 0774/10) and all of the participants provided written informed consent.

All of the patients enrolled into this study had a diagnosis of asthma according to the Global Initiative for Asthma (GINA/2012), which included a compatible clinical history of asthma and at least one pulmonary function test showing airway obstruction with reversibility [39]. The inclusion criteria consisted of patients with asthma, aged 18–75 years, who had never smoked and had no other associated lung disease or cognitive disability that would prevent their participation. We excluded patients who had other respiratory chronic diseases (except rhinitis), were smokers or ex-smokers, were not able to perform pulmonary function tests, or presented other severe uncontrolled chronic diseases. During follow-up, we also excluded patients with discordant results of skin prick (SPT) and serum-specific IgE (sIgE) results. A flow chart describing patients' enrollment is shown in Figure 1.

Study Design

Patients were prospectively followed from 2012 to 2014. They were recruited in a sequential way during their first clinic visit in 2012. Data collection, including lung function and laboratory and skin tests, was performed from 2012 to 2013 by only one physician (P.T.) with expertise in the management of asthma patients. Patients were asked regarding triggers of asthma exacerbations, the age of onset of asthma symptoms, personal and family histories of atopic diseases (asthma, rhinitis, allergic conjunctivitis, and atopic dermatitis), and their history of hypersensitivity reactions to NSAID. The patients' medical records were also reviewed to collect relevant information.

All patients with asthma underwent investigation regarding sensitization to relevant aeroallergens in the city of São Paulo to characterize the diagnosis of atopy. Both in vivo (SPT) and in vitro (sIgE measurements) tests were performed. The SPT was performed in duplicate for the following allergens: *Dermatophagoides pteronyssinus*, *Blomia tropicalis*, *Cladosporium herbarum*, *Alternaria alternata*, *Penicillium notatum*, *Aspergillus fumigatus*, *Canis familiaris*, *Felis domesticus*, *Lolium perenne*, *Periplaneta Americana*, and *Blattella germanica*. Histamine (10 mg/ml) was used as a positive control, and 0.9% physiological saline was used as a negative control. The test was considered positive when the mean wheal diameter induced by the allergen was 3 mm larger than the mean wheal diameter of the negative control. Standardized allergen extracts from International Pharmaceutical Immunology (IPI-ASAC, São Paulo, Brazil) were used. Peripheral venous blood samples were collected from the patients for measurements of serum total IgE, blood eosinophil count, and sIgE to *D. pteronyssinus*, *B. tropicalis*, fungi, and animal epithelia (cat, dog, horse, and cow) using the ImmunoCAP system (Pharmacia Diagnostics, Uppsala, Sweden). Specific IgE values greater than or equal to 0.35 kU/L were considered positive.

Table 1. Characteristics of the study participants

	Allergic asthma (<i>n</i> = 56)	Nonallergic asthma (<i>n</i> = 53)	Control (<i>n</i> = 297)	<i>p</i> value	Cramer's <i>V</i>	
					Cohen's <i>d</i>	95% CI
Age, years	44.14±14.45	57.09±12.15	43.03±17.55	<0.001 ^a		
Females, <i>n</i> (%)	44 (78.57)	46 (86.79)	114 (38.38)	<0.001 ^e		
Age at the onset of symptoms	12.80±11.82	25.38±19.79		<0.001 ^d	0.777	0.383 to 1.170
FEV ₁ , % predicted	65.46 (14.22)	71.64 (15.33)		0.031 ^b	0.418	0.035 to 0.801
Eosinophil count, cells/mm ³	0.29±0.24	0.30 (0.23)		0.875 ^c	0.024	−0.355 to 0.403
Serum immunoglobulin E, IU/mL	523.60±819.70	160.00±352.50		<0.001 ^d	0.569	0.180 to 0.958

Values are presented as means ± SD unless otherwise stated. FEV₁, forced expiratory volume in 1 s. ^a Kruskal-Wallis test. ^b Student *t* test. ^c Wilcoxon-Mann-Whitney test. ^d Brunner-Munzel test; ^e Pearson χ^2 test.

The patients were divided into 2 groups according to clinical history and the results of a SPT and serum sIgE measurement. Only patients with concordant results from in vivo and in vitro sensitization tests (both SPT and sIgE positive or both SPT and sIgE negative) were analyzed and divided into these 2 groups, while patients with discordant results were excluded. The allergic asthma group consisted of patients with asthma, a history of asthma attacks triggered by aeroallergens (upper or lower airway symptoms immediately after contact with dust or domestic animals), positive results on the SPT for at least one of the tested allergens (wheal diameter at least 3 mm larger than the negative control wheal), and positive specific IgE for any of the tested allergens (sIgE levels greater than or equal to 0.35 kU/L). The nonallergic asthma group consisted of patients with asthma, without a history of asthma attacks triggered by aeroallergens, with negative results on the SPT, and with specific IgE levels lower than 0.35 kU/L for all of the tested allergens (Fig. 1).

Clinical and laboratory data, including HLA genotyping, from the allergic and nonallergic asthma groups, were assessed and compared. Finally, we included a third group, which was considered a control group only for the HLA analysis. It consisted of deceased organ donors from the blood bank of DNA sample typing of the Laboratory of Immunology of the Heart Institute of the Hospital das Clínicas da FMUSP. Clinical data from these individuals are confidential, but almost all of them died from external causes such as trauma or violence. They usually did not suffer from severe chronic or acute diseases, as their organs are considered healthy to be transplanted into other patients. However, it is possible that some of them presented mild asthma with the same frequency as in the general population [~10% in adolescents according to the International Study of Asthma and Allergies in Childhood (ISAAC) – recent data] [40]. This group was not completely matched to asthmatic patients in terms of age or gender, but it could be considered as a sample of healthy individuals from the general Brazilian population. So, allergic and nonallergic asthma groups were compared with each other for all of the outcomes of interest, including HLA genotyping, and they were also compared with the control group in the HLA analysis.

HLA Typing

HLA genotyping for class I (A, B, and C) and class II (DR, DQ, and DP) loci was performed in all of the participants in the 3 study groups. Genomic DNA was extracted from the peripheral blood of

all of the participants using a QIAmp® DNA Blood Kit (Qiagen, Valencia, Calif., USA) according to the manufacturer's instructions. HLA typing for the HLA-A, HLA-B, and HLA-DR loci of deceased organ donors was performed via the polymerase chain reaction sequence-specific primer method using a LABType SSP Kit (One Lambda, Canoga Park, CA, USA) according to the manufacturer's instructions. HLA typing for the HLA-A, HLA-B, HLA-C, HLA-DQ, HLA-DR, and HLA-DP loci of patients with asthma, as well as typing of the HLA-C, HLA-DQ, and HLA-DP loci of the deceased organ donors, was performed via the polymerase chain reaction sequence-specific oligonucleotide method using a LABType SSO Kit (One Lambda) according to the manufacturer's instructions.

Statistical Analysis

The clinical variables of asthma were compared between groups using the Fisher exact test for categorical data and the Student *t* test (normal residuals, homoscedastic case), the Wilcoxon-Mann-Whitney test (nonnormal residuals, same scale parameter), and the Brunner-Munzel test (nonnormal residuals, different scale parameters) for numerical variables [41]. Cramer's *V* and Cohen's *d* effect sizes with their respective 95% CI were calculated.

The frequencies of HLA alleles were compared between the 3 groups using the Pearson χ^2 test or the Fisher exact test. The same test was used to compare pairs of groups, and the *p* values were adjusted using the Holm method (sequential Bonferroni). Cramer's *V* was used as the effect size measure, and a 95% bootstrap CI based on 2,000 bootstrap replications was reported [42]. A decision tree classifier was used to predict the group association based on HLA alleles using the Classification and Regression Trees algorithm [43]. The decision tree was pruned based on a cross-validation of 10 subsamples to avoid overfitting, and a confusion matrix was calculated to assess its quality. In addition, a second tree that excluded the control subjects was adjusted using the same methodology. To perform a statistical analysis of HLA genotyping, we tried to use a proportion of 3 controls to 1 asthmatic patient.

Finally, the association between NSAID and each HLA allele was assessed using the Pearson χ^2 test or the Fisher exact test with OR used to measure the effect. Wilcoxon-Mann-Whitney tests with Cohen's *d* effect sizes were used to analyze the association with IgE. All analyses were performed with R 3.1.2, and the significance level was set at 5%.

Table 2. Information regarding participants in the allergic and nonallergic asthma groups

	Allergic asthma (<i>n</i> = 56)	Nonallergic asthma (<i>n</i> = 53)	<i>p</i> value ^a	Cramer's <i>V</i>	
				estimate	95% CI
Personal history			0.034	0.255	0.089–0.380
Rhinitis	41 (73.21)	49 (92.45)			
Atopic dermatitis	5 (8.93)	1 (1.89)			
Rhinoconjunctivitis	10 (17.86)	3 (5.66)			
Family history			0.266	0.193	0.035–0.314
Asthma	24 (42.86)	26 (49.06)			
None	21 (37.50)	14 (26.42)			
Rhinitis	6 (10.71)	3 (5.66)			
Rhinitis + asthma	5 (8.93)	10 (18.87)			
Inhaled corticosteroid dose			0.001	0.337	0.131–0.480
Low	3 (5.36)	4 (7.55)			
Moderate	8 (14.29)	23 (43.40)			
High	45 (80.36)	26 (49.06)			
NSAID			<0.001	0.437	0.260–0.595
No	46 (82.14)	21 (39.62)			
Yes	10 (17.86)	32 (60.38)			

Values are presented as numbers (%) unless otherwise stated. NSAID, nonsteroidal anti-inflammatory drugs. ^a Fisher exact test.

Table 3. Frequency of HLA class I and II antigens and comparison between groups

	Allergic asthma (<i>n</i> = 56)	Nonallergic asthma (<i>n</i> = 53)	Controls (<i>n</i> = 297)	<i>p</i> value	Allergic asthma × nonallergic asthma	Allergic asthma × controls	Nonallergic asthma × controls	Cramer's <i>V</i>	
								estimate	95% CI
A*02	17 (30.36)	28 (52.83)	116 (39.06)	0.052 ^a	0.052	0.218	0.121	0.104	0.060–0.142
B*42	4 (7.14)	2 (3.77)	5 (1.68)	0.040 ^b	0.679	0.116	0.574	0.117	0.018–0.229
B*48	0 (0.00)	3 (5.66)	2 (0.67)	0.031 ^b	0.223	1.000	0.079	0.157	0.046–0.310
C*17	6 (10.71)	3 (5.66)	10 (3.38)	0.040 ^b	0.854	0.083	0.854	0.120	0.022–0.237
DPA1*03	7 (12.50)	4 (7.55)	4 (2.56)	0.013 ^b	0.391	0.026	0.229	0.159	0.073–0.226
DPB1*105	7 (12.50)	3 (5.66)	5 (3.21)	0.036 ^b	0.644	0.049	0.644	0.159	0.035–0.285

Values are presented as numbers (%) unless otherwise stated. HLA, human leukocyte antigens. ^a Pearson χ^2 test; ^b Fisher exact test, post hoc adjusted using the Holm method (sequential Bonferroni).

Results

The present study included 406 participants: 109 patients with asthma who were divided into an allergic group (*n* = 56) and a nonallergic group (*n* = 53), and 297 deceased donors of solid organs who constituted the control group of the HLA typing analysis. The mean age of the patients, the percentage of females, and the mean age at onset of asthma symptoms were significantly different between the two asthma phenotypes (*p* < 0.001), as shown in Table 1.

Analysis of the personal history of atopic diseases revealed that rhinitis was highly prevalent in both groups of patients with asthma. However, atopic dermatitis and rhinoconjunctivitis were specifically associated with allergic asthma (*p* = 0.034). Regarding the family history of atopic diseases, no significant difference was observed between the groups. A suggestive history of intolerance to NSAID was more prevalent in the nonallergic asthma group (*p* < 0.001), as shown in Table 2.

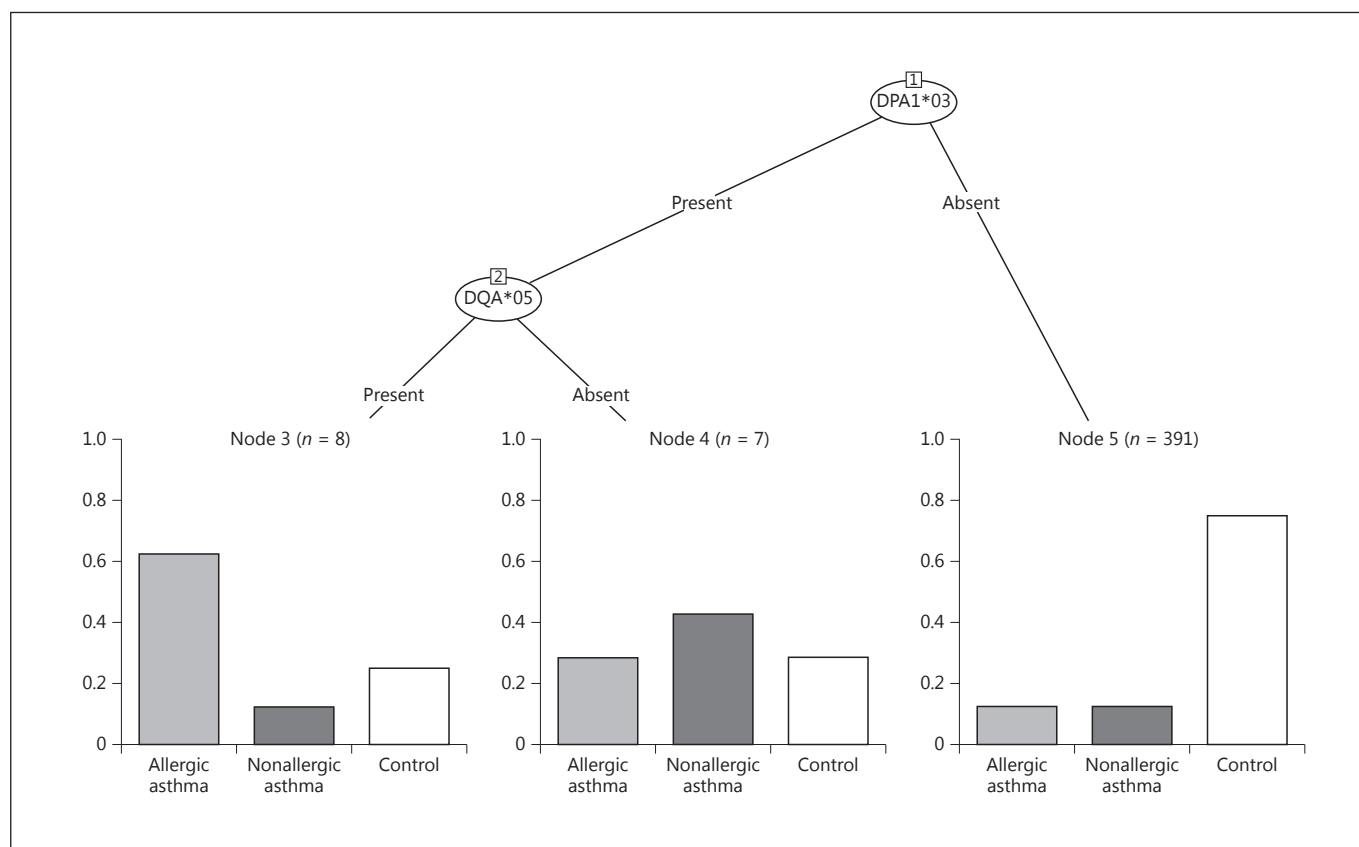


Fig. 2. Decision tree analysis among the 3 groups of this study.

All patients with asthma were on drug therapy. We found that 80.36% of the patients in the allergic group and 49.06% of the patients in the nonallergic group were using high doses of inhaled corticosteroids ($p < 0.001$) according to the GINA classification [39]. The severity of airflow obstruction was assessed via a pulmonary function test (Brazilian Guidelines for Pulmonary Functional Tests) [44], which demonstrated that bronchial obstruction was more severe among patients with allergic asthma than those with nonallergic asthma ($p = 0.031$; Table 1). Patients with allergic asthma presented higher mean serum IgE levels than those with nonallergic asthma ($p < 0.001$), but the mean blood eosinophil counts were similar between the asthma groups (Table 1).

HLA genotyping for class I (A, B, and C) and class II (DQ, DR, and DP) loci was performed in all of the participants, except for one deceased organ donor in whom the stored genetic material did not allow analysis of the HLA-C locus. In a comparison of the 3 groups, the HLA-B*42 ($p = 0.040$), HLA-C*17 ($p = 0.040$), HLA-DPA1*03 ($p = 0.013$), and HLA-DPB1*105 ($p = 0.036$) alleles were

associated with allergic asthma. In contrast, the HLA-B*48 allele ($p = 0.031$) was more frequent among patients with nonallergic asthma (Table 3).

A comparison between the allergic asthma and control groups alone revealed that the HLA-DPA1*03 and HLA-DPB1*105 alleles were associated with the allergic phenotype. As shown in Table 3, no association was observed when the nonallergic and control groups were analyzed separately. Analysis of the HLA-A*02 allele frequency showed a borderline p value ($p = 0.052$) in both asthma phenotypes.

The decision tree analysis of the 3 groups revealed that the simultaneous presence of the HLA-DPA1*03 and DQA*05 alleles was associated with allergic asthma. The nonallergic phenotype was associated with the presence of the HLA-DPA1*03 allele and the absence of the HLA-DQA*05 allele. The absence of the HLA-DPA1*03 allele was more frequent in the control group (Fig. 2). In this analysis, the positive predictive values for the allergic asthma, nonallergic asthma, and control groups were 62.5, 42.9, and 74.9%, respectively.

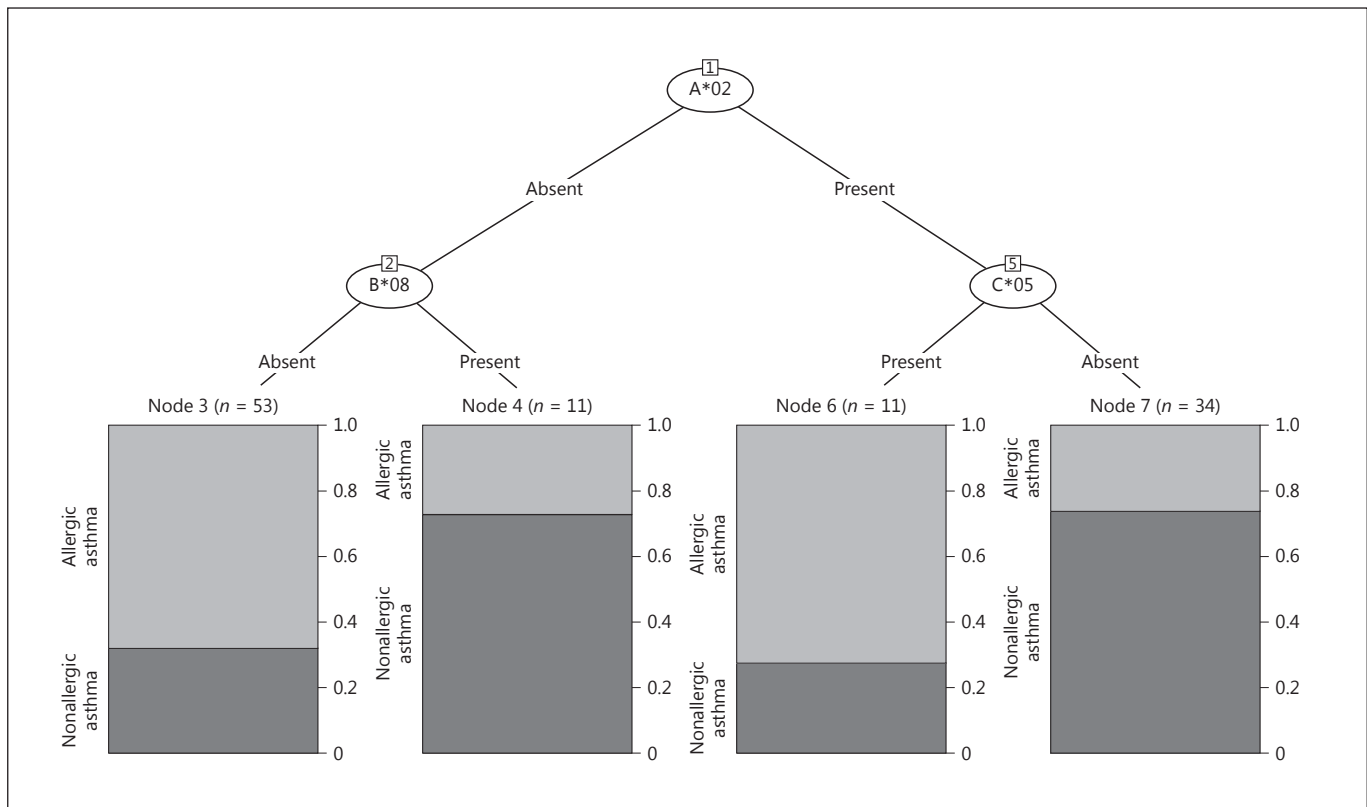


Fig. 3. Decision tree analysis between the allergic and nonallergic asthma groups.

The decision tree analysis of the allergic asthma and nonallergic asthma groups alone, excluding the control group, showed that the simultaneous presence of the HLA-A*02 and HLA-C*05 alleles, as well as the absence of the HLA-A*02 and HLA-B*08 alleles, was associated with allergic asthma. In contrast, the absence of the HLA-A*02 allele and the presence of HLA-B*08 allele, as well as the presence of the HLA-A*02 allele and the absence of the HLA-C*05 allele, was associated with the nonallergic asthma phenotype (Fig. 3). This analysis had a sensitivity of 78.6 and 62.3% and a specificity of 62.3 and 78.6% for the allergic and nonallergic phenotypes, respectively.

The HLA-B*40 and HLA-DRB1*04 alleles were more frequent, while the HLA-A*68, HLA-B*58, and HLA-DRB1*13 alleles were less frequent, among the asthmatic patients with a suggestive history of intolerance to NSAID than in those who tolerated NSAID (Table 4). The presence of the HLA-A*30 and HLA-C*17 alleles and the absence of the HLA-A*02, HLA-B*38, HLA-B*44, HLA-DRB1*16, and HLA-DQB1*03 alleles were statistically associated with elevated serum IgE levels, as shown in Table 5.

Table 4. Analysis of the participants with asthma with and without a suggestive history of intolerance to NSAID

	Intolerance to NSAID		<i>p</i> value	OR	95% CI
	no	yes			
A*68	12 (17.90)	2 (4.80)	0.046 ^a	0.23	0.02–1.13
B*40	2 (3.00)	7 (16.70)	0.026 ^b	6.39	1.14–66.32
B*58	7 (10.40)	0 (0.00)	0.042 ^b		
DRB1*04	9 (13.40)	13 (31.00)	0.027 ^a	2.86	1.00–8.56
DRB1*13	26 (38.80)	7 (16.70)	0.014 ^a	0.32	0.10–0.87

Values are presented as numbers (%) unless otherwise stated. NSAID, nonsteroidal anti-inflammatory drugs. ^a Pearson χ^2 test. ^b Fisher exact test.

Discussion

Asthma is one of the most common chronic disorders in the world. The disease is associated with a high morbidity and may be fatal if not properly treated. Asthma should be considered a syndrome or at least a disease with

Table 5. Mean serum IgE levels and association with HLA class I and II antigens

	IgE				<i>p</i> value ^a	Cohen's <i>d</i>	95% CI
	absent HLA		present HLA				
	<i>n</i>	mean ± SD	<i>n</i>	mean ± SD			
A*02	63	492.38±825.18	45	147.19±185.39	<0.001	0.537	0.144 to 0.930
A*30	95	305.88±531.46	13	660.38±1,253.98	0.036	0.542	−0.048 to 1.131
B*38	106	354.89±666.38	2	12.50±4.95	0.036	0.516	−0.898 to 1.930
B*44	83	393.85±732.82	25	198.16±294.78	0.031	0.297	−0.156 to 0.750
C*17	99	348.32±689.47	9	351.11±184.25	0.029	0.004	−0.685 to 0.693
DRB1*16	103	362.78±674.44	5	55.40±50.23	0.039	0.465	−0.444 to 1.373
DQB1*03	51	406.94±738.32	57	296.31±586.63	0.029	0.167	−0.215 to 0.549

IgE, immunoglobulin E; HLA, human leukocyte antigens. ^a Wilcoxon-Mann-Whitney test.

different phenotypes [45]. Association studies should be conducted in this context. An association between a genetic risk factor and a particular disease phenotype might not appear when one addresses the population of all asthmatic patients [46, 47]. Identification of a probable association between the HLA system and allergic and nonallergic asthma phenotypes is important to better understand the pathophysiology of the disease, particularly the nonallergic phenotype, and to improve clinical decisions based on the mechanism of the disease [48, 49].

The mean age at the onset of asthma symptoms was different between the studied phenotypes; it was earlier among patients with allergic asthma and later among those with nonallergic asthma, which coincides with the literature. A predominance of females was observed in both asthma phenotypes and it was statistically higher in the nonallergic asthma group. Several studies have shown that asthma is more prevalent and severe among women [47].

An association between nonallergic asthma and intolerance to nonsteroidal anti-inflammatory drugs and nasal polyposis has been described in the literature [50]. The present study confirms this finding and shows that intolerance to nonsteroidal anti-inflammatory drugs is more common among patients with nonallergic asthma than among patients with allergic asthma. However, the problem is also relevant in the allergic phenotype.

Studies have shown that the nonallergic asthma phenotype is characterized by a greater severity of the disease [6, 51]. However, allergic asthma patients in the present study showed more severe bronchial obstruction and used higher doses of inhaled corticosteroids than nonallergic patients.

IgE is an antibody produced in response to allergen exposure, although high levels can also be found in other conditions such as hyper IgE syndrome, allergic bronchopulmonary aspergillosis, drug reactions, and intestinal parasites [52]. The use of eosinophilia and total serum IgE levels as biomarkers for asthma in Brazil is limited because intestinal parasitoses are endemic in the country. We observed, as expected, higher serum IgE levels among patients with allergic asthma and similar blood eosinophil counts between the phenotypes.

Personal and family histories of atopic diseases are characteristic of patients with allergic asthma. In the present study, the presence of atopic dermatitis and rhinoconjunctivitis were associated with allergic asthma. However, the presence of rhinitis and a family history of atopy was not different between the asthma phenotypes.

Studies associating the HLA system (class I and class II) with asthma are inconsistent and report that these loci confer both an increased risk for and protection from the disease. The limitations of these studies include their small sample size, the fact that different asthma phenotypes are ignored, the diversity of HLA class I and II alleles selected in the studies, the existence of unknown alleles, ethnic characteristics of the populations, and the methodology used in HLA genotyping.

Studies have evaluated the possible association between the HLA type and the age at onset of asthma symptoms. A higher frequency of HLA-A10 and a lower frequency of HLA-B5 antigens were observed among patients who developed the disease before the age of 17 years, as was a higher frequency of the HLA-B8 antigen in patients with a later onset [53]. In contrast, a study in Croatian children demonstrated that HLA-B8 was associ-

ated with the allergic phenotype [54]. Another study showed an increased frequency of HLA-B12 in patients with allergic asthma and a lower frequency of the HLA-A3, HLA-B7, and HLA-DR2 antigens in patients with nonallergic asthma [55]. The GABRIEL consortium analyzed a sample of 10,365 participants with asthma and 16,110 controls and identified an association of the HLA-DQ region with later-onset disease (age 16 years or older) [56]. A study of a Venezuelan population suggested that the HLA-DRB1*11:01 HLA-DQA1*05:01 HLA-DQB1*03:01 haplotype is associated with allergic asthma, which confers susceptibility to the development of asthma with mite sensitization. Furthermore, it concluded that HLA-C7 could play a protective role in the development of the allergic phenotype [34].

HLA-DQ was the first locus reported to be associated with asthma [57]. The TENOR study reported an association of the RAD50-IL13 and HLA-DR/DQ regions with asthma susceptibility among patients with severe or difficult-to-treat asthma [58]. There are few studies available in the literature assessing the association between asthma and the HLA-DP locus. The first genome-wide association study in Asian populations revealed that the HLA-DPA1*0201 and HLA-DPB1*0901 alleles were associated with pediatric asthma [59]. A study in patients with allergic asthma in a mulatto population demonstrated a statistically lower frequency of the HLA-DPB1*0401 allele, suggesting a protective factor for the disease [29].

The only study similar to ours analyzed the frequency of HLA class I and II (excluding the HLA-DP typing) loci in patients with allergic and nonallergic asthma. A higher frequency of the HLA-A1, HLA-B7, and HLA-DQB1*0302 antigens and a lower frequency of the HLA-B18 antigen were observed among patients with nonallergic asthma, but all of the findings were not statistically significant [35].

In terms of aspirin hypersensitivity, we found a higher frequency of the alleles HLA-B*40 and HLA-DRB1*04 between asthmatic patients and intolerance to NSAID (Table 4). HLA-DRB1*04 has already been associated with aspirin-induced asthma [37], and HLA-DPB1 has been reported to be the most relevant marker of this phenotype [60]. We did not find an association with this allele in our cohort. However, we did not expect to find an association between a class I genotype of HLA (HLA-B*40) and this particular Th2-high phenotype of asthma. It is possible that other unknown mechanisms are involved in the pathogenesis of aspirin-induced asthma in which Th1 cells could play a role. Nevertheless, as we did not per-

form aspirin provocation tests in all patients, it is possible that this finding is not completely reliable.

Analysis of the HLA class I locus in the present study revealed that the HLA-B*42 and HLA-C*17 alleles were associated with the allergic asthma phenotype, while the HLA-B*48 allele was associated with the nonallergic phenotype. The allergic phenotype was associated with the presence of the HLA-A*02 and HLA-C*05 haplotypes and the absence of the HLA-A*02 and HLA-B*08 haplotypes. In contrast, nonallergic asthma was associated with the absence of the HLA-A*02 haplotype and the presence of HLA-B*08 haplotype, as well as the presence of the HLA-A*02 haplotype and the absence of the HLA-C*05 haplotype.

Analysis of the HLA class II antigens revealed that the frequency of the HLA-DPA1*03 and HLA-DPB1*105 haplotypes was higher in patients with allergic asthma. The presence of the HLA-DPA1*03 DQA*05 haplotype was associated with allergic asthma, while the presence of the HLA-DPA1*03 allele and the absence of the HLA-DQA*05 allele were associated with nonallergic asthma. In addition, we observed new HLA genotypes associations in asthmatic patients with intolerance to NSAID and with elevated serum IgE levels.

Our study has several limitations. First of all, there was a small sample of patients with asthma despite the fact that they were well separated between allergic and nonallergic phenotypes. Moreover, it is possible that an ideal control group, such as healthy blood donors, for example, should have been used instead of organ donors who had already died. However, HLA genotyping is quite expensive and difficult to perform and the present study had no financial support. This “blood bank” of DNA sampling from our laboratory represents in some way the general Brazilian population. Finally, we did not perform aspirin provocation tests in all patients in order to confirm hypersensitivity, which may have been overestimated.

The Brazilian population resulted from mixing between European Caucasians, Native Indians, and Africans. Analysis of HLA system polymorphisms in mixed populations may show different frequencies of alleles and haplotypes of HLA compared to other racial and ethnic groups, which may influence the associations between the HLA system and diseases. Thus, knowledge and characterization of the HLA system and subsequent associations with diseases in different human populations have great value, especially in a country with a great racial diversity.

The present study is innovative and relevant due to two main attributes. First, strict criteria were used to

identify and compare the allergic and nonallergic asthma phenotypes. Second, associations not previously described between HLA and asthma were identified. It is likely that several alleles/haplotypes are involved in asthma depending on the disease phenotype and ethnic characteristics of the study population. Furthermore, the genetic predisposition of asthma is polygenic and new studies on large populations are needed to confirm the role of HLA as a protective or risk factor in the several disease phenotypes.

Disclosure Statement

I confirm that this paper was read and approved by all of the coauthors and that no conflicts of interests exist regarding its publication.

Author Contributions

All of the authors made substantial contributions to conception of this study, drafted this article and revised it critically for important intellectual content, and approved the final version for publication.

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