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Association between HLA genes and dust mite sensitivity in a Brazilian population

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ARTICLE INFO

Article history:

Received 21 June 2016

Revised 20 October 2016

Accepted 20 October 2016

Available online xxx

Keywords:

Mites

Sensitive

Genetic association

HLA

Disease susceptibility

ABSTRACT

Background: Type I hypersensitivity, also known as IgE-mediated allergy, is a complex, multifactorial condition whose onset and severity are influenced by both genetic and environmental factors. Mite allergens stimulate the production of humoral response (IgE), especially in children, which is closely involved in atopic asthma and rhinitis.

Objective: This study aimed to investigate the association between HLA class I (-A, -B, and -C), and HLA class II (-DRB1) genes in individuals sensitive to dust mites (*Dermatophagoides farinae*, *Dermatophagoides pteronyssinus*, or *Blomia tropicalis*) and mite-insensitive controls.

Methods: 396 participants were grouped as mite-sensitive and mite-insensitive according to immediate hypersensitivity as determined by skin-prick tests, and to HLA genotyping by polymerase chain reaction-sequence specific oligonucleotide (PCR-SSO).

Results: After chi-square heterogeneity testing no significant differences were observed in HLA-A, B, and C genes, except for the HLA-DRB1 locus, which, showed a negative association for *DRB1*04*, between mite-sensitive and mite-insensitive individuals. In high resolution, *DRB1*04:11* allele was significantly different from all other results ($P = 0.0042$, $OR = 0.26$, and $95\%CI = 0.09–0.70$). The analysis stratified by etiologic agent confirmed these associations.

Conclusion: Our results suggest a possible association between HLA-DRB1 genes and hypersensitivity to dust mites.

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1. Introduction

The development of advanced techniques in molecular biology has allowed the study of structural and functional genomics, the genetics of certain populations, and evolutionary genetics, studies that can help to gain a better understanding of the role of each individual gene in the physiology of the immune response. In humans, the immune system, has evolved to protect the host from a universe of pathogens, is composed of a large number of highly polymorphic genes, i.e., the presence of multiple alleles at a locus, being found at frequencies greater than 5% in certain populations, which results in a complex system with important consequences

for the pathophysiology of several diseases that affect humans [1–3].

Allergy is one of these diseases. It produces immune responses to environmental antigens, causing tissue inflammation and organ dysfunction. The etiology of atopy involves genetic factors that are still poorly understood. Sensitization occurs when antigen presenting cells (APCs) of predisposed individuals process allergens (proteins) and present the resulting peptides within HLA molecules to the allergen-specific CD4⁺ T cells. The latter produce cytokines that induce a class switch in B cells to IgE antibodies, which bind to the high affinity FcεRI on mast cells and basophils (rich in vasoactive substances). Upon a second contact, the allergen cross-links these IgE antibodies, and the effector cells react immediately to release preformed inflammatory substances within seconds. The ability of these allergens to make individuals become sensitized and respond by producing IgE antibodies varies and is determined by the individual's genetic makeup and by cultural and environmental factors [4,5].

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Both the prevalence and severity of allergic respiratory diseases have increased over the past 5 decades, represent global health problems for all age groups, particularly among children. This is possibly due to environmental changes and increased exposure to allergens. More than 50,000 species of mite allergens have been described, and house dust mites, such as *Dermatophagoides pteronyssinus* (*D. pteronyssinus*) and *Dermatophagoides farinae* (*D. farinae*), are the most common among the known atopic allergens. These mites have been found in house dust samples worldwide, being more prevalent in warm, humid climates [6,7,4].

The human leukocyte antigen (HLA) system is a complex of several loci in the major histocompatibility complex (MHC) region of chromosome 6 in humans, whose encoded products are found in cell-surface molecules and subdivided into class I and II according to their molecular structure, tissue distribution, and biological function. This system is characterized by a high degree of polymorphism, uneven distribution of allele frequencies in the population, and prevalence of typical alleles in certain populations. HLA molecules are responsible for the presentation of peptides to immunocompetent T cells. In addition to interacting with natural killer cell receptors the HLA molecules are considered to play a key role in the adaptive immune response, since they are involved in the onset, maintenance, protection, or predisposition to certain diseases [8–10].

In a previous study, allelic and genotypic associations in pro-inflammatory (*IL1A*⁻⁸⁸⁹ and *IL2*⁻³³⁰), and anti-inflammatory (*IL4*⁻⁵⁹⁰, *IL4RA*⁺¹⁹⁰² and *IL10*⁻⁵⁹²) cytokine variants were observed in allergic individuals [11]. Considering that HLA molecules are also immune response genes and connect innate and adaptive immune responses, the prior results led us to the current study. In addition, no other study has yet distinguished between the three most frequent allergen sources in tropical regions.

It is worth mentioning that in the southern hemisphere, no database exists for the sensitization to the most important allergen sources, namely the different species of dust mites.

To date, some studies have investigated the relationship between HLA genes and sensitivity to dust mites (Table 1), however, the importance of these associations remains unclear. Considering that the identification of candidate genes in mite-sensitive individuals could reveal mechanisms of interaction between allergens and hosts, the aim of the present study was to conduct a genetic association study to investigate markers of immune response in HLA class I (-A, -B, and -C), and HLA class II (-DRB1) genes in individuals sensitive to dust mites (*D. farinae*, *D. pteronyssinus*, or *Blomia tropicalis*) compared to mite-insensitive controls.

2. Methods

2.1. Ethical considerations

The study was approved by the Research Ethics Committee of Universidade Estadual de Maringá (protocol No. 412.420/2013) and conducted in accordance with the provisions of the Declaration of Helsinki. All participants provided written informed consent prior to their inclusion in the study. In the case of minors, under 18 years of age, written consent was obtained from their parents/legal guardian.

2.2. Participants, inclusion and exclusion criteria

The inclusion criteria adopted by this cross-sectional study included 343 children (individuals under 14 years of age) and 53 adolescents and educators (individuals over 14 years of age) from 3 public institutions located in the city of Maringá (Paraná, southern Brazil: latitude 23°25'31"S and longitude 51°56'19"W) who

Table 1

Overview of HLA-DRB1 data associated with sensitization to house dust mites.

HLA alleles groups	OR	P	Investigation
<i>DRB1*04</i>	<1	0.002	Development of a sensitization to house dust mite <i>D. pteronyssinus</i> [22]
	<1	0.010*	The risk of developing asthma in citrus red mite-exposed adults [24]
	>1	0.006	HLA-DR genotypes and immunoglobulin E responses to common major allergens [43]
<i>DRB1*07</i>	>1	0.009	Development of a sensitization to house dust mite <i>D. pteronyssinus</i> [22]
	>1	0.010*	The risk of developing asthma in citrus red mite-exposed adults [24]
<i>DRB1*11</i>	>1	0.020*	The influence of the MHC class I and class II genes in the susceptibility to atopic asthma in a Venezuelan population [44]
<i>DRB1*13</i>	>1	0.021	The association between tumor necrosis factor, HLA-DR alleles, and IgE-mediated asthma in Taiwanese adolescents [39]
<i>DRB1*14</i>	<1	0.020	Development of a sensitization to house dust mite <i>D. pteronyssinus</i> [22]
HLA allele <i>DRB1*08:03:02</i>	>1	0.009	Association between HLA class II genes alleles and Allergic rhinitis in house dust mites sensitive Chinese subjects [45]
HLA haplotypes <i>DRB1*11:01</i> <i>DQA1*05:01</i> <i>DQB1*03:01</i>	>1	<0.010*	The influence of the MHC class I and class II genes in the susceptibility to atopic asthma in a Venezuelan population [44]
<i>DRB*01/DRB1*03/DRB1*11</i>	>1	<0.010	The relevance of HLA-class II molecules to the regulation of immune response to the mite allergen <i>D. pteronyssinus</i> and to clinical atopic disorders [46]
<i>DQB*03:03/*05:03</i> , <i>DRB1*02/DRB1*07</i> , and <i>DPB*04:02</i>	<1	< or =0.01	The relevance of HLA-class II molecules to the regulation of immune response to the mite allergen <i>D. pteronyssinus</i> and to clinical atopic disorders [46]

* Corrected P value; OR = odds ratio.

voluntarily agreed to participate in the study. Individuals were randomly invited to participate, and 396 agreed to enter the study.

The exclusion criteria included, consanguineous individuals and Asian descent. Whereas to the ethnical group, considering the rejection of subjects of Asian origin, all other participants were considered to be white Brazilians, bearing in mind that, the Brazilian population is heterogeneous as well as highly mixed.

2.3. Allergy testing, and sample collection

Each participant's sensitized status was determined by skin prick testing. At least 1 of the 3 tested mite allergens (*D. farinae*, *D. pteronyssinus*, and *Blomia tropicalis*) had to be positive. A positive response was defined as presence of erythema ranging from <15 mm (+) to >30 mm (++++). For each individual, a negative and a positive control parameter were tested. Reactions were read and noted by two specialists, 10–15 min after the reagents were applied, according to the manufacturer's instructions (Anthygenus™, Rio de Janeiro, RJ, Brazil). The mite-insensitive group consisted of individuals with no history of allergy and who showed no simultaneous reaction to the 3 allergens, serving as healthy controls.

Peripheral blood samples (10 mL) were collected from all individuals into tubes containing EDTA as anticoagulant. DNA was then extracted using the commercial kit (Biopur™, Reinach, Switzerland), according to the manufacturer's instructions. Genomic DNA (OD ratio 260/280 of 1.6–1.9) was adjusted to a concentration of 20–50 ng/μL.

2.4. HLA Class I and II Genotyping

HLA class I (HLA-A, -B, and -C) and class II (HLA-DRB1) were genotyped by polymerase chain reaction-sequence specific oligonucleotide (PCR-SSO) using the commercial kit SSO LabType™ (One Lambda, Canoga Park, CA, USA).

The amplified product was denaturated and hybridized with microbeads linked to specific oligonucleotide probes for determination of HLA genotypes. Complementary products were detected by chemiluminescence and analyzed using the Luminex™ flow cytometer (LabScan™ 100), and the results were interpreted using HLA Fusion™, version 3.1 (One Lambda, Canoga Park, CA, USA).

2.5. Ambiguities

One of the alternatives to solve the ambiguities found in the PCR-SSO technique, was the use of PCR-SSP method (in house) followed by PCR-SBT. When one of the ambiguities suggested unusual genotypes, it was adopted as a criterion, the inclusion of the most common genotypes, based on our database of bone marrow donors. Most ambiguities of the HLA-B locus were resolved by specific primers of Bw4 group provided by the manufacturer. In addition, allele and haplotype frequencies obtained were compared using the Allele Frequencies Net Database (AFND) (<http://www.allelefrequencies.net>).

2.6. Statistical analysis

The high levels of polymorphism and strong linkage disequilibrium (LD) associated with the HLA loci must be taken specifically for this purpose when analyzed data for disease association studies, in order to avoid confounding results. Population analyses, including calculations of alleles and genotype frequencies, tests for fit to expectations under Hardy–Weinberg equilibrium, and estimations of haplotype frequencies, were the initial steps in this study involving immunogenetic data [12].

According to Hollenbach et al. [13], the second step was to use a contingency table to test the difference, or independence, of frequency distributions for categorical variables. This table was constructed utilizing raw counts data. The approach in this type of analysis for highly polymorphic immunogenetic data was to first examine heterogeneity in overall allele frequency distributions in cases vs. controls for a particular locus, using a $2 \times k$ row by column ($r \times c$) contingency table, in OpenEpi software (version 3.01), available on: <http://www.openepi.com>.

For rare alleles, there is a particular difficulty, although the χ^2 test has been the standard approach for testing fit at the overall locus-level, this test can lead to false acceptance or rejection of the null hypothesis when the expected genotype counts in a contingency table are small (sparse cells), in accordance with Hollenbach et al. [13], the χ^2 test is inappropriate if any of the expected counts are less than one or if the expected counts are less than five in more than 20% of all cells in a contingency table. Albeit Yates' correction for continuity is sometimes applied to account for numerous sparse cells, this may be overly conservative, and the preferred method is to combine low frequency alleles into a single category, often referred to as the “binned” category.

Therefore, the conservative approach was respected, combining alleles with expected value of less than five in cases or controls into the binned category prior to calculation of the χ^2 statistic.

The degrees of freedom (DF) for the goodness of fit χ^2 analysis were calculated from the number of alleles with expected values in cases and controls of five or greater, plus the combined category, -1 (i.e., $k - 1$). A p-value was obtained by comparing the test statistic to the χ^2 distribution for the appropriate degrees of freedom.

Whereas to results that were significant at the overall locus level, additional testing were used 2×2 contingency tables for each allele (expected counts of five or more only) against all other alleles.

In Relative Predispositional Effects (RPE) analysis [14], by means of the OpenEpi software (version 3.01), alleles, haplotypes, or genotypes with the strongest predisposing (PRE) or protective (PRO) effects were sequentially removed from the analysis, until there is no further significant heterogeneity detected either overall or for an individual allele, to reveal the relative predispositional effects of the HLA alleles that are associated with the disease.

Allele frequencies were determined by direct counting and compared using a 2×2 contingency table. The significance of the differences was evaluated using two-tailed Fisher's exact test [15], and P-values less than 0.05 were considered significant, obtained by use of the Epi Info™ (version 7.0). Odds ratio (OR) was calculated, with a 95% confidence interval (95%CI) according to Woolf [16], using Epi Info™ (version 7.0). Hardy–Weinberg equilibrium was tested using the method described by Guo and Thompson [17] with the Arlequin software, version 3.5 [18].

Because the gametic phase is unknown, maximum-likelihood estimates of haplotype frequencies were obtained from multilocus genotype data and computed using the expectation-maximization (EM) algorithm [19], also using the Arlequin software, version 3.5 [18].

3. Results

Of 396 individuals, 45.5% (180/396) were male and 54.5% (216/396) were female. A total of 249 (63%) were included as cases (individuals who were atopic to at least one mite) and 147 (37%) as controls (non-atopic individuals). There was no difference in sex distribution between cases and controls ($P = 0.2509$).

Regarding age, only 13% (53/396) of the total participants were adults, the group of sensitized individuals had a mean (SD) age of 12.5 (6.9) years (range, 4–48 years), while non-sensitized controls had a mean (SD) age of 11.5 (5.8) years (range, 5–37 years). There was no difference in age between the two groups ($P = 0.1408$).

Overall, 56% tested positive for *D. farinae*, 43% for *D. pteronyssinus*, 37% for *Blomia tropicalis*, and 29% for the 3 mites simultaneously.

The observed and expected frequencies between genotypes for loci A, B, C, and DRB1 showed no deviation from Hardy–Weinberg equilibrium in cases or controls, with P-values greater than 0.05.

The frequencies of HLA-A, -B, -C, and -DRB1 alleles were calculated and no differences were found between the populations studied, except for the DRB1 locus, which showed a statistically significant difference for the DRB1*04 allele group (8.8% vs. 14.6%, $P = 0.0135$, OR = 0.56, and 95%CI = 0.36–0.88), when compared to sensitized individuals to at least one of the dust mites and controls (Supplementary Table 1).

As shown in Table 2 (Supplementary Table 2), when HLA-DRB1 high-resolution allele frequencies were compared, the most frequent allele in both case and control groups was DR*07:01, with 13.4% vs. 12.2%. However, the DRB1*04:05 and DRB1*04:11 alleles was significantly less frequent in cases (1.0% vs. 3.1%, $P = 0.0481$,

Table 2

Allele frequencies of HLA-DRB1 in individuals sensitized to at least one of the following dust mites: *Dermatophagoides farinae*, *Dermatophagoides pteronyssinus*, and *Blomia tropicalis*, and controls.

HLA	Cases		Controls		P	OR	95%CI
	n = 249	%	n = 147	%			
DRB1*01:01	31	6.2	15	5.1	0.6374	1.23	0.65–2.32
DRB1*01:02	20	4.0	8	2.7	0.4273	1.49	0.65–3.44
DRB1*03:01	41	8.2	28	9.5	0.6021	0.85	0.51–1.41
DRB1*04:04	6	1.2	9	3.1	0.1017	0.38	0.13–1.09
DRB1*04:05	5	1.0	9	3.1	0.0481*	0.32	0.10–0.96
DRB1*04:11	6	1.2	13	4.4	0.0067*	0.26	0.09–0.70
DRB1*07:01	67	13.4	36	12.2	0.6629	1.11	0.72–1.71
DRB1*08:01	12	2.4	8	2.7	0.8169	0.88	0.35–2.18
DRB1*08:04	7	1.4	10	3.4	0.0758	0.40	0.15–1.07
DRB1*09:01	7	1.4	10	3.4	0.0758	0.40	0.15–1.07
DRB1*10:01	8	1.6	8	2.7	0.3032	0.58	0.21–1.57
DRB1*11:01	43	8.6	17	5.8	0.1651	1.53	0.86–2.75
DRB1*11:02	9	1.8	8	2.7	0.4494	0.65	0.25–1.72
DRB1*11:04	12	2.4	8	2.7	0.8169	0.88	0.35–2.18
DRB1*13:01	39	7.8	12	4.1	0.0503	1.99	1.02–3.87
DRB1*13:02	19	3.8	9	3.1	0.6920	1.25	0.56–2.81
DRB1*14:01	12	2.4	8	2.7	0.8169	0.88	0.35–2.18
DRB1*15:01	25	5.0	22	7.5	0.1637	0.65	0.36–1.18
DRB1*15:03	11	2.2	13	4.4	0.0884	0.48	0.21–1.10
Binned	118	23.9	43	14.6	0.0018*	1.83	1.24–2.68

HLA = human leukocyte antigen; OR = odds ratio; P = two-tailed Fisher's exact test; 95%CI = 95% confidence interval; Binned = sum of values < 5 in cases or controls; * = P value < 0.05.

OR = 0.32, and 95%CI = 0.10–0.96 and 1.2% vs. 4.4%, $P = 0.0067$, OR = 0.26, and 95%CI = 0.09–0.70), respectively.

For the DRB1 locus, the frequencies of HLA alleles were compared for each mite species (*D. farinae*, *D. pteronyssinus*, and *Blomia tropicalis*) separately. All species differed statistically, and were associated as a protective to hypersensitivity.

The DRB1*04 allele group had a negative association with allergy to *D. farinae* and *D. pteronyssinus* ($P = 0.0339$, OR = 0.60 and $P = 0.0166$, OR = 0.53), respectively (Tables 3 and 4). There was also a negative association between allergy to *Blomia tropicalis* and both the DRB1*04 allele group ($P = 0.0089$ and OR = 0.49) and the DRB1*15 allele group ($P = 0.0295$ and OR = 0.54) (Table 5).

For the chi-square heterogeneity test, there were no significant heterogeneity in allele counts in HLA class I. However, for the HLA class II, the DRB1 locus (in high-resolution) was the only one to show significant differences between cases and controls ($\chi^2 = 45.26$ e $P = 0.0006$) (Table 6).

In Relative Predispositional Effects (RPE) analysis, no significant heterogeneity was detected when DRB1*04:11 was removed,

revealing the relative predispositional effects of this HLA allele associated with the disease. The results of DRB1*04:11 in the chi-square test using 2×2 contingency table were: 1.2% vs. 4.4%, $P = 0.0042$, OR = 0.26, and 95%CI = 0.09–0.70.

In relation to haplotypes, no significant difference was found between mite-sensitive and mite-insensitive individuals, and the most frequent haplotype was HLA-A*01 B*08 C*07 DRB1*03, with 2.2% vs. 2.4%, respectively (Supplementary Table 3).

The genotype and allele frequencies of HLA-C killer cell immunoglobulin-like receptor (KIR) ligands, called C1 and C2, were also compared; however, no significant difference was found.

Finally, analyses based on a series of 2×2 tests requires corrections for multiple comparisons (usually utilizing the Bonferroni method), with a correction factor minimally equivalent to the number of alleles tested. However, when significance of the association of all individual alleles was assessed with a priori knowledge of overall heterogeneity at the locus, it was not necessary to correct for multiple comparisons in subsequent 2×2 tests intended to identify the allele(s) with significant contributions to the overall deviation at the locus [13,20].

Table 3

Allele group frequencies of HLA-DRB1 in individuals sensitized to *Dermatophagoides farinae* and controls.

HLA	Cases		Controls		P	OR	95%CI
	n = 223	%	n = 147	%			
DRB1*01	52	11.7	23	7.8	0.1055	1.55	0.92–2.60
DRB1*03	42	9.4	31	10.5	0.6165	0.88	0.54–1.43
DRB1*04	42	9.4	43	14.6	0.0339*	0.60	0.38–0.95
DRB1*07	61	13.7	36	12.2	0.6563	1.13	0.73–1.76
DRB1*08	33	7.4	24	8.2	0.7783	0.89	0.51–1.55
DRB1*09	7	1.6	10	3.4	0.1323	0.45	0.17–1.20
DRB1*10	5	1.1	8	2.7	0.1508	0.40	0.13–1.25
DRB1*11	60	13.5	34	11.6	0.4991	1.18	0.75–1.86
DRB1*13	59	13.2	26	8.8	0.0771	1.57	0.96–2.55
DRB1*14	19	4.3	10	3.4	0.6993	1.26	0.57–2.75
DRB1*15	42	9.4	38	12.9	0.1469	0.70	0.43–1.11
DRB1*16	13	2.9	7	2.4	0.8178	1.23	0.48–3.12
Binned	11	2.5	4	1.4	0.4255	1.83	0.57–5.81

HLA = human leukocyte antigen; OR = odds ratio; P = two-tailed Fisher's exact test; 95%CI = 95% confidence interval; Binned = sum of values < 5 in cases or controls; * = P value < 0.05.

Table 4Allele group frequencies of HLA-DRB1 in individuals sensitized to *Dermatophagoides pteronyssinus* and controls.

HLA	Cases		Controls		P	OR	95%CI
	n = 174	%	n = 147	%			
DRB1*01	35	10.1	23	7.8	0.3374	1.31	0.75–2.28
DRB1*03	36	10.3	31	10.5	1.0000	0.97	0.58–1.62
DRB1*04	29	8.3	43	14.6	0.0166*	0.53	0.32–0.87
DRB1*07	50	14.4	36	12.2	0.4857	1.20	0.75–1.90
DRB1*08	25	7.2	24	8.2	0.6572	0.87	0.48–1.55
DRB1*09	5	1.4	10	3.4	0.1194	0.41	0.13–1.22
DRB1*10	6	1.7	8	2.7	0.4264	0.62	0.21–1.82
DRB1*11	48	13.8	34	11.6	0.4092	1.22	0.76–1.95
DRB1*13	46	13.2	26	8.8	0.1022	1.57	0.94–2.61
DRB1*14	11	3.2	10	3.4	1.0000	0.92	0.38–2.21
DRB1*15	36	10.3	38	12.9	0.3230	0.77	0.47–1.26
DRB1*16	12	3.4	7	2.4	0.4893	1.46	0.56–3.76
Binned	9	2.6	4	1.4	0.4004	1.92	0.58–6.31

HLA = human leukocyte antigen; OR = odds ratio; P = two-tailed Fisher's exact test; 95%CI = 95% confidence interval; Binned = sum of values < 5 in cases or controls; * = P value < 0.05.

Table 5Allele group frequencies of HLA-DRB1 in individuals sensitized to *Blomia tropicalis* and controls.

HLA	Cases		Controls		P	OR	95%CI
	n = 148	%	n = 147	%			
DRB1*01	37	12.5	23	7.8	0.0760	1.68	0.97–2.91
DRB1*03	33	11.1	31	10.5	0.8948	1.06	0.63–1.78
DRB1*04	23	7.8	43	14.6	0.0089*	0.49	0.28–0.83
DRB1*07	41	13.9	36	12.2	0.6253	1.15	0.71–1.86
DRB1*08	21	7.1	24	8.2	0.6449	0.85	0.46–1.57
DRB1*09	7	2.4	10	3.4	0.4730	0.68	0.25–1.83
DRB1*11	44	14.9	34	11.6	0.2741	1.33	0.82–2.15
DRB1*13	36	12.2	26	8.8	0.2269	1.42	0.83–2.43
DRB1*14	12	4.1	10	3.4	0.8286	1.20	0.51–2.82
DRB1*15	22	7.4	38	12.9	0.0295*	0.54	0.31–0.93
DRB1*16	7	2.4	7	2.4	1.0000	0.99	0.34–2.86
Binned	13	4.4	12	4.1	1.0000	1.07	0.48–2.40

HLA = human leukocyte antigen; OR = odds ratio; P = two-tailed Fisher's exact test; 95%CI = 95% confidence interval; Binned = sum of values < 5 in cases or controls; * = P value < 0.05.

Table 6

Heterogeneity test by chi-square for HLA class I and II.

Locus	Chi-square (χ^2)	DF	P
HLA-A	16.69	12	0.1615
HLA-B	24.19	22	0.3374
HLA-C	16.61	13	0.2176
HLA-DRB1 ^a	45.26	19	0.0006*

DF = degrees of freedom; P = Uncorrected p-value from the chi-square test of heterogeneity; * = P value < 0.05.

^a High-resolution.

4. Discussion

Allergy is a complex phenomenon with manifestations involving the skin, digestive tract, and even the eyes (among many other sites). It is considered a multifactorial condition whose onset and severity are influenced by both genetic and environmental factors [21].

The production of IgE antibodies against allergens from house dust mites is closely involved in the development of atopic asthma and rhinitis. Although several family-based studies have confirmed the participation of genetic control in this response, the mechanism that explains this relationship remains to be fully elucidated [22].

Ninety percent of asthmatic children show signs of IgE-mediated allergy to inhaled proteins, described as allergens [23]. It is known that these specific IgE responses to allergens provide

useful models for understanding the genetic factors that control immune responses [24].

Solé et al. [25], in a study on the epidemiology of asthma in the Brazilian pediatric population, reported that the mean prevalence of asthma in Brazilian adolescents remains among the highest averages in the world. The study points out that living in a rural environment is a protective factor in the development of asthma and allergic diseases. Another nationwide survey, which included children and adolescents treated at different Brazilian allergy centers, showed positivity rates of 66.7% for *D. pteronyssinus*, 64.5% for *D. farinae*, and 55.2% for *Blomia tropicalis* [26].

In our previous studies, we found a strong association with single nucleotide polymorphisms (SNPs) of cytokines in mite-sensitive individuals. In particular, IL-4 [11], which is involved in IgE production of B cells, is associated with mite-sensitivity. The immunological reaction triggered by the interaction of HLA and peptide may alter the presentation to CD4+ T cells and consequently the activation of certain cytokines.

HLA class I and II genes have been revealed as candidates for involvement in the etiology of allergic respiratory diseases, such as asthma [27,28]. Initially, a relationship between HLA class II molecules and IgE responses was observed in patients sensitized to allergens. Later, different studies involving epidemiological investigation revealed a number of HLA-DR and DQ genes that were able to restrict the recognition of allergens from house dust mites [22].

Other studies already reported the involvement of HLA-DRB1 allele group in individuals with diagnosis of rhinoconjunctivitis

and/or bronchial asthma and a positive skin prick test [29]. Another finding, described that *HLA-DRB1*03:01* plays an important function in Th2-predominant immune response to house dust mite [30], as well as *HLA-DRB1*07* with susceptibility to and *DRB1*04* and *DRB1*14* as protective factors against the development of allergy to *D. pteronyssinus* [22].

In the present study, when mite species were evaluated individually, the *DRB1*04* allele group showed a protective effect for all of 3 mites (*D. farinae*, *D. pteronyssinus* and *Blomia tropicalis*). When high-resolution allele frequencies were compared, the *DRB1*04:11* allele was significantly different. In our Brazilian population, the frequency of this allele is approximately 1.7%, being a typical allele in Amerindian populations in South America. High frequencies of this allele have been observed among the Guarani-M'yba (26.8%) of Brazil, the Ache (74.1%) of Paraguay, and the Yucpa (60.2%) of Venezuela. Considering that this allele would be more advantageous to the survival of individuals exposed to greater environmental adversity, it is possible to make an association between the effect of the diversifying selection pressure exerted by pathogens, the overdominance effect of HLA genes, and frequency-dependent selection [31].

In a Korean research with 178 atopic individuals (to *D. pteronyssinus*) and 99 non-atopic controls, Kim et al. [22] suggested a strong negative association (27.5% vs. 45.5%, $P = 0.002$) for *DRB1*04*. In another study, with the same researchers, investigating Koreans with asthma induced by the citrus red mite, negative association was also observed for the same allele group (19.8% vs. 40.8%, $P_c = 0.01$) [24]. In Hungary, Bede et al. [32] conducted a case-control study involving 57 patients with bronchial asthma caused by mite allergy and two control groups, one with 57 non-atopic children and the other with 45 mite-insensitive asthmatic children, to examine the frequencies of *DRB1*07* and *DRB1*04* alleles groups. The authors found a negative association for the *DRB1*04* allele group when frequencies were compared between cases and non-atopic controls ($P \leq 0.01$ and $OR = 0.21$). These findings are consistent with the present results, since, according to OR values, the *DRB1*04* allele group was associated as a protective gene for the disease.

Parapanissiou et al. [33], investigated 60 Greek children with allergic bronchial asthma due to mite sensitivity (*D. farinae* and *D. pteronyssinus*) and 125 controls, they observed an important factor in susceptibility to asthma with sensitivity to mites related to *DRB1*04*.

Concerning diverging results, it is understood that the allelic group ranges a great variety of alleles, which only through high-resolution could the association be determined. Moreover, it is to be considered that our results displayed association to the *DRB1*04:11* allele which is only found in Amerindian populations.

In addition to being associated with protection against allergic respiratory disease, the *DRB1*04* allele group is also an important marker of susceptibility to other diseases, such as rheumatoid arthritis. Studies conducted in Korea ($P < 0.00005$) [34], in China [35], in Japan [36], and in Hungary [37] have well elucidated the association of this gene with susceptibility to rheumatoid arthritis.

Soriano et al. [38], in a study conducted in Spain after 26 outbreaks of asthma caused by the inhalation of soybean dust, affecting 688 individuals, raised the hypothesis that a genetic determinant, in this case the HLA class II molecules, could have influenced the susceptible individuals. Indeed, their results showed that the risk of epidemic asthma was particularly associated with the *DRB1*13* gene ($P < 0.02$). In the present study, this gene also showed a tendency towards positive association with mite allergy but the allele *DRB1*13:01* was excluded after the heterogeneity test was applied.

The HLA-DR alleles have also been investigated by researchers from China, who evaluated the association between tumor necrosis

factor, HLA-DR molecules, and IgE-mediated asthma in 80 Taiwanese adolescents with asthma and 69 without asthma. The *DRB1*13* gene showed a positive association (11.3% vs. 1.5%; $P = 0.021$) and was related to both high dust-mite-specific IgE and high total serum IgE, especially for *D. farinae* [39], findings that are in contrast to the results observed in the current study.

The *DRB1*15*, in the present study, suggested a resistance effect in the development of hypersensitivity to *Blomia tropicalis*, but after the heterogeneity test, it was disregarded. This gene was also investigated in a case-control study involving 103 children with asthma and 152 controls, and the results showed a negative association with asthma (35% vs. 80%, $P = 0.003$, $OR = 0.46$, and 95% $CI = 0.28–0.78$) [40]. Reinforcing such evidence, Kalpaklioglu and Turan [41] revealed that for this given gene, a relation of resistance between cockroach sensitivity and cutaneous reactivity to aeroallergens ($P < 0.001$). In addition, *DRB1*15* was also reported to play a protective role in the development of asthma in the Asian Indian population, since this is the most common class II allele in this population, which may explain the lower incidence of asthma compared to other regions of the world [42].

Finally, this study allows us to expand our horizons in the understanding of the immune response and sensitivity to dust mite, in the future we may be able to analyze the complex interaction of the effector sites of the HLA molecule with different peptides. Other genetic markers of immune response may also be investigated, such as MICA and KIR genes, to better understand the relationship between gene expression and allergy.

For the elucidation of the obtained data, we suggest further studies in different populations, for example, the Amerindians, using high-resolution methodology for defining alleles. Our results could be the basis for a three-dimensional study of analysis of HLA epitopes with the peptides, and the interrelation with clinical and laboratory aspects (IgE and cytokines).

In conclusion, our results support previously reported associations between the *DRB1*04* allele group and protection against developing IgE-mediated allergy to *Dermatophagoides farinae*, *Dermatophagoides pteronyssinus*, and *Blomia tropicalis*.

Funding

This work was supported by Araucária Foundation [Agreement 268/14 Protocol 37803-24/2012 Project Number].

Acknowledgments

We would like to thank all the participants for their contribution to the study.

Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.humimm.2016.10.014>.

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Supplementary Table 1. Allele group frequencies of HLA-A, -B, -C, and -DRB1 in individuals sensitized to at least one of the following dust mites: *Dermatophagoides farinae*, *Dermatophagoides pteronyssinus*, and *Blomia tropicalis*, and controls.

HLA	Cases		Controls		<i>P</i>	OR	95%CI
	n= 249	%	n= 147	%			
<i>A*01</i>	47	9.4	30	10.2	0.7117	0.91	0.56-1.48
<i>A*02</i>	118	23.7	75	25.5	0.6073	0.90	0.64-1.26
<i>A*03</i>	45	9.0	29	9.9	0.7060	0.90	0.55-1.48
<i>A*11</i>	31	6.2	13	4.4	0.3367	1.43	0.73-2.78
<i>A*23</i>	25	5.0	15	5.1	1.0000	0.98	0.50-1.89
<i>A*24</i>	47	9.4	21	7.1	0.2952	1.35	0.79-2.31
<i>A*25</i>	5	1.0	6	2.0	0.3455	0.48	0.14-1.60
<i>A*26</i>	15	3.0	9	3.1	1.0000	0.98	0.42-2.27
<i>A*29</i>	28	5.6	14	4.8	0.7430	1.19	0.61-2.30
<i>A*30</i>	27	5.4	12	4.1	0.4972	1.34	0.67-2.70
<i>A*31</i>	19	3.8	16	5.4	0.2879	0.68	0.34-1.36
<i>A*32</i>	19	3.8	6	2.0	0.2088	1.90	0.75-4.82
<i>A*33</i>	23	4.6	10	3.4	0.4655	1.37	0.64-2.93
<i>A*34</i>	4	0.8	6	2.0	0.1862	0.38	0.10-1.38
<i>A*36</i>	3	0.6	2	0.7	1.0000	0.88	0.14-5.32
<i>A*68</i>	33	6.6	25	8.5	0.3269	0.76	0.44-1.31
<i>A*74</i>	9	1.8	5	1.7	1.0000	1.06	0.35-3.20
HLA	n= 249	%	n= 147	%	<i>P</i>	OR	95%CI
<i>B*07</i>	30	6.0	28	9.5	0.0893	0.60	0.35-1.04
<i>B*08</i>	24	4.8	16	5.4	0.7380	0.87	0.45-1.68

<i>B*13</i>	13	2.6	5	1.7	0.4691	1.54	0.54-4.39
<i>B*14</i>	33	6.6	16	5.4	0.5448	1.23	0.66-2.28
<i>B*15</i>	42	8.4	29	9.9	0.5209	0.84	0.51-1.38
<i>B*18</i>	32	6.4	10	3.4	0.0721	1.95	0.94-4.02
<i>B*27</i>	17	3.4	9	3.1	0.8397	1.11	0.49-2.54
<i>B*35</i>	58	11.6	29	9.9	0.4815	1.20	0.75-1.92
<i>B*37</i>	5	1.0	3	1.0	1.0000	0.98	0.23-4.14
<i>B*38</i>	7	1.4	2	0.7	0.4967	2.08	0.42-10.08
<i>B*39</i>	12	2.4	6	2.0	0.8100	1.18	0.44-3.19
<i>B*40</i>	26	5.2	13	4.4	0.7345	1.19	0.60-2.35
<i>B*41</i>	3	0.6	4	1.4	0.4336	0.43	0.09-1.97
<i>B*42</i>	8	1.6	9	3.1	0.2062	0.51	0.19-1.35
<i>B*44</i>	54	10.8	26	8.8	0.3950	1.25	0.76-2.05
<i>B*45</i>	9	1.8	3	1.0	0.5502	1.78	0.47-6.64
<i>B*46</i>	0	0	1	0.3	0.3712	0	Undef
<i>B*47</i>	0	0	1	0.3	0.3712	0	Undef
<i>B*48</i>	2	0.4	2	0.7	0.6302	0.58	0.08-4.20
<i>B*49</i>	8	1.6	9	3.1	0.2062	0.51	0.19-1.35
<i>B*50</i>	16	3.2	5	1.7	0.2551	1.91	0.69-5.29
<i>B*51</i>	33	6.6	29	9.9	0.1313	0.64	0.38-1.09
<i>B*52</i>	21	4.2	8	2.7	0.3310	1.57	0.68-3.60
<i>B*53</i>	13	2.6	6	2.0	0.8170	1.28	0.48-3.42
<i>B*54</i>	1	0.2	0	0	1.0000	Undef	Undef
<i>B*55</i>	2	0.4	4	1.4	0.2020	0.29	0.05-1.60
<i>B*56</i>	3	0.6	0	0	0.2988	Undef	Undef

<i>B*57</i>	14	2.8	10	3.4	0.6709	0.82	0.36-1.87
<i>B*58</i>	11	2.2	9	3.1	0.4872	0.71	0.29-1.74
<i>B*59</i>	0	0	1	0.3	0.3712	0	Undef
<i>B*81</i>	1	0.2	1	0.3	1.0000	0.58	0.03-9.46
HLA	n= 249	%	n= 147	%	<i>P</i>	OR	95%CI
<i>C*01</i>	13	2.6	11	3.7	0.3951	0.68	0.30-1.55
<i>C*02</i>	36	7.2	23	7.8	0.7803	0.91	0.53-1.58
<i>C*03</i>	42	8.4	26	8.8	0.8957	0.94	0.56-1.58
<i>C*04</i>	81	16.3	40	13.6	0.3578	1.23	0.81-1.85
<i>C*05</i>	32	6.4	11	3.7	0.1431	1.76	0.87-3.56
<i>C*06</i>	47	9.4	22	7.5	0.3648	1.28	0.75-2.18
<i>C*07</i>	97	19.5	71	24.1	0.1267	0.75	0.53-1.07
<i>C*08</i>	35	7.0	20	6.8	1.0000	1.03	0.58-1.83
<i>C*12</i>	32	6.4	12	4.1	0.1993	1.61	0.81-3.18
<i>C*14</i>	12	2.4	8	2.7	0.8169	0.88	0.35-2.18
<i>C*15</i>	20	4.0	21	7.1	0.0672	0.54	0.28-1.02
<i>C*16</i>	38	7.6	15	5.1	0.1873	1.53	0.83-2.84
<i>C*17</i>	11	2.2	12	4.1	0.1872	0.53	0.23-1.21
<i>C*18</i>	2	0.4	2	0.7	0.4746	0.58	0.08-4.20
HLA	n= 249	%	n= 147	%	<i>P</i>	OR	95%CI
<i>DRB1*01</i>	56	11.2	23	7.8	0.1408	1.49	0.89-2.48
<i>DRB1*03</i>	48	9.6	31	10.5	0.7133	0.90	0.56-1.45
<i>DRB1*04</i>	44	8.8	43	14.6	0.0135*	0.56	0.36-0.88
<i>DRB1*07</i>	67	13.5	36	12.2	0.6629	1.11	0.72-1.71
<i>DRB1*08</i>	36	7.2	24	8.2	0.6772	0.87	0.51-1.50

<i>DRB1*09</i>	7	1.4	10	3.4	0.0758	0.40	0.15-1.07
<i>DRB1*10</i>	8	1.6	8	2.7	0.3032	0.58	0.21-1.57
<i>DRB1*11</i>	68	13.7	34	11.6	0.4427	1.20	0.77-1.87
<i>DRB1*12</i>	12	2.4	4	1.4	0.4350	1.79	0.57-5.60
<i>DRB1*13</i>	67	13.5	26	8.8	0.0528	1.60	0.99-2.58
<i>DRB1*14</i>	21	4.2	10	3.4	0.7051	1.25	0.58-2.69
<i>DRB1*15</i>	48	9.6	38	12.9	0.1574	0.71	0.45-1.12
<i>DRB1*16</i>	16	3.2	7	2.4	0.6622	1.36	0.55-3.34

HLA= human leukocyte antigen; OR= odds ratio; *P*= two-tailed Fisher's exact test; 95%CI= 95% confidence interval; Undef= undefined.

Supplementary Table 2. Allele frequencies of HLA-DRB1 in individuals sensitized to at least one of the following dust mites: *Dermatophagoides farinae*, *Dermatophagoides pteronyssinus*, and *Blomia tropicalis*, and controls.

HLA	Cases		Controls		<i>P</i>	OR	95%CI
	n= 249	%	n= 147	%			
<i>DRB1*01:01</i>	31	6.2	15	5.1	0.6374	1.23	0.65-2.32
<i>DRB1*01:02</i>	20	4.0	8	2.7	0.4273	1.49	0.65-3.44
<i>DRB1*01:03</i>	4	0.8	0	0	0.3026	Undef	Undef
<i>DRB1*01:04</i>	1	0.2	0	0	1.0000	Undef	Undef
<i>DRB1*03:01</i>	41	8.2	28	9.5	0.6021	0.85	0.51-1.41
<i>DRB1*03:02</i>	7	1.4	3	1.0	0.7519	1.38	0.35-5.38
<i>DRB1*04:01</i>	9	1.8	2	0.7	0.2269	2.68	0.57-12.52
<i>DRB1*04:02</i>	4	0.8	3	1.0	0.7146	0.78	0.17-3.53
<i>DRB1*04:03</i>	7	1.4	1	0.3	0.2697	4.17	0.51-34.12
<i>DRB1*04:04</i>	6	1.2	9	3.1	0.1017	0.38	0.13-1.09
<i>DRB1*04:05</i>	5	1.0	9	3.1	0.0481*	0.32	0.10-0.96
<i>DRB1*04:06</i>	0	0	1	0.3	0.0516	0	Undef
<i>DRB1*04:07</i>	5	1.0	2	0.7	1.0000	1.48	0.28-7.68
<i>DRB1*04:08</i>	2	0.4	2	0.7	0.6302	0.58	0.08-4.20
<i>DRB1*04:09</i>	0	0	1	0.3	0.3712	0	Undef
<i>DRB1*04:11</i>	6	1.2	13	4.4	0.0067*	0.26	0.09-0.70
<i>DRB1*07:01</i>	67	13.4	36	12.2	0.6629	1.11	0.72-1.71
<i>DRB1*08:01</i>	12	2.4	8	2.7	0.8169	0.88	0.35-2.18
<i>DRB1*08:02</i>	12	2.4	4	1.4	0.4350	1.79	0.57-5.60
<i>DRB1*08:03</i>	5	1.0	1	0.3	0.4207	2.97	0.34-25.56
<i>DRB1*08:04</i>	7	1.4	10	3.4	0.0758	0.40	0.15-1.07

<i>DRB1*08:07</i>	0	0	1	0.3	0.3712	0	Undef
<i>DRB1*09:01</i>	7	1.4	10	3.4	0.0758	0.40	0.15-1.07
<i>DRB1*10:01</i>	8	1.6	8	2.7	0.3032	0.58	0.21-1.57
<i>DRB1*11:01</i>	43	8.6	17	5.8	0.1651	1.53	0.86-2.75
<i>DRB1*11:02</i>	9	1.8	8	2.7	0.4494	0.65	0.25-1.72
<i>DRB1*11:03</i>	4	0.8	1	0.3	0.6563	2.37	0.26-21.32
<i>DRB1*11:04</i>	12	2.4	8	2.7	0.8169	0.88	0.35-2.18
<i>DRB1*12:01</i>	12	2.4	4	1.4	0.4350	1.79	0.57-5.60
<i>DRB1*13:01</i>	39	7.8	12	4.1	0.0503	1.99	1.02-3.87
<i>DRB1*13:02</i>	19	3.8	9	3.1	0.6920	1.25	0.56-2.81
<i>DRB1*13:03</i>	8	1.6	3	1.0	0.7547	1.58	0.41-6.01
<i>DRB1*13:05</i>	1	0.2	2	0.7	0.5589	0.29	0.02-3.25
<i>DRB1*14:01</i>	12	2.4	8	2.7	0.8169	0.88	0.35-2.18
<i>DRB1*14:02</i>	7	1.4	2	0.7	0.4967	2.08	0.42-10.08
<i>DRB1*14:04</i>	1	0.2	0	0	1.0000	Undef	Undef
<i>DRB1*14:06</i>	1	0.2	0	0	1.0000	Undef	Undef
<i>DRB1*15:01</i>	25	5.0	22	7.5	0.1637	0.65	0.36-1.18
<i>DRB1*15:02</i>	12	2.4	3	1.0	0.1897	2.93	0.67-8.55
<i>DRB1*15:03</i>	11	2.2	13	4.4	0.0884	0.48	0.21-1.10
<i>DRB1*16:01</i>	6	1.2	4	1.4	1.0000	0.88	0.24-3.15
<i>DRB1*16:02</i>	10	2.0	3	1.0	0.3912	1.98	0.54-7.28

HLA= human leukocyte antigen; OR= odds ratio; *P*= two-tailed Fisher's exact test; 95%CI= 95% confidence interval; Undef= undefined.

Supplementary Table 3. Haplotype frequencies of HLA in individuals sensitized to at least one of the following dust mites: *Dermatophagoides farinae*, *Dermatophagoides pteronyssinus*, and *Blomia tropicalis*, and controls.

Haplotype	Cases n= 249	%	Controls n= 147	%	<i>P</i>
A*01-B*08-C*07-DRB1*03	11	2.2	7	2.4	ns
A*29-B*44-C*16-DRB1*07	10	2.0	3	1.0	ns
A*02-B*35-C*04-DRB1*11	10	2.1	0	0	ns
A*33-B*14-C*08-DRB1*01	8	1.6	3	1.0	ns
A*03-B*07-C*07-DRB1*15	6	1.2	6	2.0	ns
A*11-B*35-C*04-DRB1*07	5	1.0	0	0	ns
A*02-B*44-C*05-DRB1*04	5	1.0	0	0	ns
A*02-B*13-C*06-DRB1*07	4	0.8	3	1.0	ns
A*02-B*44-C*05-DRB1*13	3	0.6	3	1.0	ns
A*02-B*15-C*07-DRB1*13	3	0.6	3	1.0	ns
A*02-B*44-C*04-DRB1*07	2	0.4	3	1.0	ns
A*24-B*44-C*16-DRB1*11	1	0.2	3	1.0	ns
A*02-B*07-C*07-DRB1*01	1	0.2	4	1.4	ns
A*68-B*40-C*03-DRB1*04	0	0	4	1.4	ns
A*68-B*15-C*03-DRB1*08	0	0	5	1.7	ns
A*24-B*35-C*04-DRB1*04	0	0	3	1.0	ns
A*02-B*52-C*07-DRB1*04	0	0	3	1.0	ns
A*02-B*51-C*15-DRB1*04	0	0	4	1.4	ns
A*02-B*49-C*07-DRB1*11	0	0	3	1.0	ns
Others	429		234		

P= two-tailed Fisher's exact test; ns= nonsignificant.