



Association between KIR genes and dust mite sensitization in a Brazilian population

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ABSTRACT

Background: Killer cell immunoglobulin-like receptors (KIRs), found on the surface of natural killer (NK) cells, play a key role in controlling the innate response. Such response depends on a series of cellular interactions between these receptors and HLA activating/inhibiting ligands. Atopic diseases have been associated with genes that regulate cytokine production and HLA genes, which may either protect or predispose to hypersensitivity. **Objective:** To verify an association study of KIR genes with sensitization to the following mites: *Dermatophagoides farinae*, *Dermatophagoides pteronyssinus*, and *Blomia tropicalis*.

Methods: A total of 341 children aged up to 14 years, were classified as mite-sensitive or mite-insensitive after undergoing a skin prick test for immediate allergic reactions. The presence/absence of KIR genes and their human leukocyte antigen (HLA) ligands was determined by polymerase chain reaction-sequence specific oligonucleotide (PCR-SSO) with the commercial kit LabType™ using Luminex™.

Results: The frequencies of KIR genes and their respective class I HLA ligands and the frequency of haplotypes were performed in sensitive and insensitive individuals, and no significant differences were found.

Conclusion: Our results suggest no influence of KIR genes on resistance/susceptibility to sensitization to dust mites.

1. Introduction

Atopic diseases, such as asthma, allergic rhinitis, atopic dermatitis, and food allergies, are caused by a number of risk factors, including genetic predisposition, age, allergens, prenatal exposure, diet, infections, seasonal effects, climate, and psychosocial factors. Some studies have shown that environmental factors have a modifying effect on the expression of different genes in atopic disease [1,2].

Chronic and allergic inflammatory diseases affect millions of people worldwide [3]. In developing countries, particularly in Latin America, the prevalence of asthma in children is high [4], and most of these children have allergic sensitization. In tropical countries, such as Brazil, mites are the main aeroallergens responsible for respiratory allergies [5]. In Thailand, a region also considered to have a tropical climate, a study showed that more than 70% of children with respiratory allergy were sensitized to mites [6]. In recent studies, it was estimated that 65–130 million individuals worldwide are sensitized against dust mites [7].

Hypersensitivity to house dust mite allergens is a usual allergic reaction. *Dermatophagoides farinae* (*D. farinae*) and *Dermatophagoides pteronyssinus* (*D. pteronyssinus*) are the most common house dust mites. These arthropods thrive in high temperatures and humidity levels [8] and are often found in beds, mattresses, carpets, and other fabric-covered items, as they feed on human skin scales [9].

Sensitization occurs when antigen presenting cells (APCs) of pre-disposed 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 [10–12].

Abbreviations: IgE, Immunoglobulin E; HLA, Human Leukocyte Antigen; KIR, Killer Cell Immunoglobulin-like Receptor; NK, Natural killer cells; PCR-SSO, Polymerase Chain Reaction-Sequence Specific Oligonucleotide; *D. pteronyssinus*, *Dermatophagoides pteronyssinus*; *D. farinae*, *Dermatophagoides farinae*; SD, Standard Deviation; OR, Odds Ratio; χ^2 , Chi-square Test; NCRs, Natural Cytotoxicity Receptors; SAPE, R-phycoerythrin conjugate

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Type I hypersensitivity reactions occur due to deviation of the host immune response toward the production of IgE antibodies, which bind to the surface of cells rich in vasoactive substances (especially histamine) and, once activated by repeated contact with the allergen, trigger inflammatory processes [13], such as vasodilation, increased vascular permeability, attraction of polymorphonuclear leukocytes, cytokine release, and stimulation of mucus secretion.

Epidemiological studies highlight an increase in the prevalence of diseases that attack the respiratory system (of allergic character). In Brazil, there was a very similar growth for two main respiratory allergic conditions, asthma and rhinitis, in recent years [14]. Solé et al. [15], in an important study involving inflammatory diseases of the airways in Brazilian children, demonstrated an average prevalence of 24.3% for active asthma and 12.6% for rhinoconjunctivitis, and the highest values were observed in the nearest centers to Ecuador. On another cross-sectional study carried out with children from the Northeast region of Brazil, the prevalence of asthma was 22.6%; of rhinitis, 43.2% and rhinoconjunctivitis, 18.7%, predominating in females [16]. It should be noted that, 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 [15], and that the diseases previously mentioned are highly prevalent in western societies [17].

Skin tests have aided in diagnosing sensitive individuals using specific allergens, whose reaction occurs immediately and the result is given in millimeters of diameter of the formed reaction. That is, this method quickly defines hypersensitivity, detects *in vivo* the presence of IgE antibodies, due to the high sensitivity and specificity, is the most widely used diagnostic tool in the field of allergies [14,18].

Natural killer (NK) cells are key components of the immune system. A major function of NK cells is to control cell interactions and death and to release cytokines. Other than their well-established role in controlling tumor activity, NK cells are now reported to play a role in many other diseases. Mandal and Viswanathan [19] reported that NK cells are a major determinant of the development of viral-associated asthma. This is related to their biological role, as NK cells contribute to the progress of T-cell-mediated allergic response during the allergen-specific sensitization phase [19], such as in house dust mite sensitization.

The function of NK cells is regulated by cell-surface receptors belonging to three major families: killer cell immunoglobulin-like receptors (KIRs), natural cytotoxicity receptors (NCRs), and C-type lectins [20]. The KIR locus in chromosomal region 19q13.4 is characterized by unusually high diversity in the numbers of both genes and their alleles [21]. The region varies in size from 100 to 350 kb as a result of structurally diverse haplotypes with duplicated segments, large deletions, and gene fusions [22,23]. As a consequence of this plasticity, the 13 distinct KIR genes (*KIR2DL1* [MIM: 604936], *KIR2DL2* [MIM: 604937], *KIR2DL4* [MIM: 604945], *KIR3DL1* [MIM: 604946], *KIR3DL2* [MIM: 604947], *KIR2DS1* [MIM: 604952], *KIR2DS2* [MIM: 604953], *KIR2DS3* [MIM: 604954], *KIR2DS4* [MIM: 604955], *KIR2DS5* [MIM: 604956], *KIR2DL5* [MIM: 605305], *KIR3DL3* [MIM: 610095], and *KIR3DP1* [MIM: 610604]) are combined in numerous ways. Haplotypes have between four and twenty KIR genes, and the most common KIR region haplotype has seven genes [24]. Each KIR gene is highly polymorphic, reaching to an extent that over 600 KIR alleles are currently defined [25], among these, there are two pseudogenes, *KIR2DP1* and *KIR3DP1* [26]. The presence or absence of certain genes results in extensive haplotype diversity and consequently high degree of polymorphism within populations. In general, based on the variability of KIR gene content, the haplotypes are divided into two primary sets, named haplotypes A and B [27].

KIRs interact with their human leukocyte antigen (HLA) ligands, which are found in almost all nucleated cells [28]. Recognition occurs specifically with one (or more) of the four epitopes of class I HLA molecules. These molecules show a dimorphism at position 80 in the $\alpha 1$ helix of the HLA-C locus, where they are classified as HLA-C1 and HLA-

C2. The receptors *KIR2DL2*, *KIR2DL3* and *KIR2DS2* recognize HLA-C1 molecules, which include the most common allelic groups (*HLA-C*01*, *C*03*, *C*07*, and *C*08*), while *KIR2DL1* and *KIR2DS1* bind to HLA-C2 molecules, with the most common allelic groups corresponding to *HLA-C*02*, *C*04*, *C*05*, and *C*06* [29]. In addition to that, the receptor *KIR3DL2* recognizes and interacts with HLA-A3 and -A11 [30]. Other ligands recognized by KIRs include HLA-Bw4 molecules, which differ from Bw6 based on the variability of their amino acids at positions 77–83 of the transcribed gene [31]. While Bw6 is found only in HLA-B epitopes, Bw4 is present in HLA-B and some HLA-A molecules [32].

Advances in the understanding of the KIR complex are extremely important primarily because of their biological function, but also because of the need for a better understanding of how these genes affect the activation and regulation of inflammatory processes. Although recent studies have demonstrated the involvement of NK cells in adverse mechanisms, little is known about the role of these cells and their receptors in atopic disease.

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 [33] and HLA-DRB1*04 molecules [34] with mite sensitivity, the aim of the present study was to investigate the association between KIR genes in sensitive to the following mites: *D. farinae*, *D. pteronyssinus*, and *Blomia tropicalis* and non-sensitive individuals.

2. Material and 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 that was obtained from their parents/legal guardian.

2.2. Study design

The sample consisted of 341 individuals, randomly selected from three public institutions in the city of Maringá (Paraná, southern Brazil: latitude 23° 25' 31" S and longitude 51° 56' 19" W), who voluntarily agreed to participate in the study. Children aged up to 14 years were included in the study. Of these, 177 (52%) were female and 164 (48%) were male. The mean (SD) age of the sample was 10 (2.1) years.

Two groups were formed, one of individuals that are sensitive to at least one of the dust mites investigated ($n = 211$), and other one with mite insensitive group ($n = 130$).

Consanguineous individuals and those of Asian descent were excluded from the study. It is known that HLA alleles have a heterogeneous distribution across racial groups. Our region has a strong Asian component due to Japanese immigration to Brazil in the 20th century. Therefore, based on information about their ancestors, individuals of Asian descent were excluded from the sample. Based on this (exclusion) criterion, all other participants were considered Brazilian whites, and ethnicity matching was not required because the Brazilian population is heterogeneous.

2.3. Biological samples

Blood samples (10 mL) were collected from all individuals into tubes containing EDTA as anticoagulant and kept frozen until extraction. Genomic DNA was extracted using the commercial kit Biopur™ (Reinach, Switzerland), according to the manufacturer's instructions, quantified using the NanoDrop™2000 UV-Vis spectrophotometer (Thermo Scientific, Waltham, MA, USA) and adjusted to a concentration of 30–50 ng/ μ L, with a purity of 1.6–1.9 (OD 260/280).

2.4. Allergy testing

The participants' allergic status was determined by skin prick testing (Anthygenus™, Rio de Janeiro, RJ, Brazil). Participants were defined as sensitive when they showed hypersensitivity to at least one of the three investigated mites: *D. farinae*, *D. pteronyssinus*, and/or *Blomia tropicalis*. Individuals who did not react to any of the tested allergen extracts served as (insensitive) controls.

Each participant underwent a skin prick test for immediate allergic reactions. The right forearm was cleaned with 70% alcohol and the allergen extracts of the three investigated mites were applied using a disposable prick lancet (3–4 cm apart), followed by a positive control (0.01% histamine chlorohydrate) and a negative control. Results were obtained 15–20 min after the test was applied and interpreted by two observers, followed by proper recording. A positive reaction was defined as the presence of red papule formation (reaction indicative of IgE), which was classified using plus signs (+) to indicate intensity [< 15 mm (+) to > 30 mm (+++)], while a negative reaction was defined as the absence of papule or erythema formation, also when the measured values of these papules or erythema were less than 15 mm, according to the standard established by the manufacturer of the test used. Based on information provided, none of the participants were taking any medication that could influence the skin test results.

Individuals insensitive to the test did not present a clinical history of allergic diseases and respiratory compromise.

2.5. Typing of KIR and HLA class I genes

The presence/absence of KIR genes and HLA class I types (-A, -B, and -C) was determined by polymerase chain reaction-sequence specific oligonucleotide (PCR-SSO) with the commercial kit LabType™ SSO (One Lambda, Canoga Park, CA, USA) using Luminex™. Each PCR product was biotinylated to be detected by streptavidin R-phycoerythrin conjugate (SAPE) and then denatured and hybridized with probes conjugated with encoded fluorescent microbeads. Complementary products were detected by chemiluminescence, which identifies phycoerythrin fluorescence intensity in each microbead, and analyzed on a flow cytometer (LabScan™ 100). The results were interpreted using HLA Fusion™ version 3.1 and Fusion Research version 3.0 (One Lambda, Canoga Park, CA, USA).

2.6. Statistical analysis

KIR genes and HLA ligands frequencies were determined by direct counting in mite-sensitive and mite-insensitive individuals. Bw4, C1 and C2 groups were defined based on information obtained from the IMGT/HLA database, according to the locus of the positions of typed genotypes. The significance of differences was evaluated using two-tailed Fisher's exact test [35], and a *P*-value less than 0.05 was considered significant using Epi Info™ (version 7.0).

The heterogeneity test proposed by Hollenbach et al. [36] was also used and calculated based on a $2 \times k$, row-by-column ($r \times c$) contingency table. *P*-values were estimated using the chi-square test (χ^2) in OpenEpi (version 3.01), available at: <http://www.openepi.com>. Based on this method, alleles frequencies with an expected value of less than 5 in cases or controls were combined into a single category, referred to as the *binned* category. The degrees of freedom for the χ^2 analysis were calculated from the number of alleles -1 (e.g., $k - 1$).

3. Results

Out of the 341 individuals, 211 (61.9%) were considered sensitive, that is, those that reacted to at least one of the three mites investigated (*D. farinae*, *D. pteronyssinus*, and/or *Blomia tropicalis*), while 130 (38.1%) were included as mite-insensitive group. The frequencies of the KIR genes *2DL1*, *2DL2*, *2DL3*, *2DL4*, *2DL5*, *2DP1*, *2DS1*, *2DS2*, *2DS3*,

Table 1

Distribution of KIR genes between individuals sensitized to at least one of the following dust mites *Dermatophagoides farinae*, *Dermatophagoides pteronyssinus*, and/or *Blomia tropicalis*, and mite-insensitive group.

Genes ¹	Mite-sensitive		Mite-insensitive		<i>P</i>
	n = 211	%	n = 130	%	
<i>2DL1</i>	207	98.1	125	96.2	ns
<i>2DL2</i>	116	55.0	77	59.2	ns
<i>2DL3</i>	186	88.2	116	89.2	ns
<i>2DL4</i>	211	100	130	100	ns
<i>2DL5</i>	114	54.0	67	51.5	ns
<i>2DP1</i>	208	98.6	128	98.5	ns
<i>2DS1</i>	80	37.9	50	38.5	ns
<i>2DS2</i>	114	54.0	70	53.8	ns
<i>2DS3</i>	55	26.1	31	23.8	ns
<i>2DS4</i>	195	92.4	125	96.2	ns
<i>2DS5</i>	80	37.0	48	36.9	ns
<i>3DL1</i>	198	93.8	126	96.9	ns
<i>3DL2</i>	211	100	130	100	ns
<i>3DL3</i>	211	100	130	100	ns
<i>3DP1</i>	211	100	130	100	ns
<i>3DS1</i>	81	38.4	51	39.2	ns

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

¹ Heterogeneity test – $\chi^2 = 0.77$, *P* = > .99 and degrees of freedom = 15.

2DS4, *2DS5*, *3DL1*, *3DL2*, *3DL3*, *3DP1*, and *3DS1* in participants sensitive to at least one mite and mite-insensitive are shown in Table 1. As expected, *2DL4*, *3DL2*, *3DL3* and *3DP1* were present in all individuals in both groups, as they are framework genes. When mite-sensitive and mite-insensitive groups were compared, using Fisher's exact test and the heterogeneity test, there was no significant difference between the two groups.

An analysis of KIR genes and their respective class I HLA ligands was performed in individuals sensitive to at least mite and controls, and the results are shown in Table 2, respectively. However, no significant differences were found between cases and controls.

Table 3 shows the distribution of KIR gene frequencies among individuals who were sensitive to a mite species vs. individuals insensitive to this species (including mite-insensitive controls). Most individuals were sensitive to *D. farinae* (*n* = 55.4%), followed by *D. pteronyssinus* (*n* = 43.0%) and *Blomia tropicalis* (*n* = 40.8%).

When comparing sensitive individuals (according to the species) with the insensitive group, using a 2×2 contingency table and heterogeneity test, no significant differences were found (Table 3).

In Table 4, frequencies of the KIR genes of the present study were compared to three other populations in the state of Paraná - Brazil: mixed population of our region (79.8% of individuals were Euro-Brazilians and 20.2% African Brazilians) [37]. Caucasoid population of Curitiba [38] and the Japanese population of Curitiba [39]. No significant difference was observed between the frequencies of KIR genes

Table 2

Frequencies of KIR genes and their ligands HLA Class I in individuals sensitized to at least one of the following dust mites *Dermatophagoides farinae*, *Dermatophagoides pteronyssinus*, and/or *Blomia tropicalis*, and mite-insensitive group.

KIR-Ligands ¹	Mite-sensitive		Mite-insensitive		<i>P</i>
	n = 211	%	n = 130	%	
<i>2DL1-C2</i>	146	69.2	90	69.2	ns
<i>2DL2-C1</i>	90	42.6	64	49.2	ns
<i>2DL3-C1</i>	152	72.0	96	73.8	ns
<i>2DS1-C2</i>	59	27.9	36	27.6	ns
<i>2DS4-Cw4</i>	61	28.9	30	23.1	ns
<i>3DL1-Bw4</i>	137	64.9	81	62.3	ns
<i>3DL2-A3A11</i>	59	27.9	34	26.1	ns

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

¹ Heterogeneity test – $\chi^2 = 2.00$, *P* = .91 and degrees of freedom = 6.

Table 3
Distribution of KIR genes between mite-sensitive (by species) and mite-insensitive group.

Genes	Sensitive to <i>D. farinae</i> ¹	Mite-insensitive	Sensitive to <i>D. pteronyssinus</i> ²	Mite-insensitive	Sensitive to <i>Blomia tropicalis</i> ³	Mite-insensitive
	n = 189 (%)	n = 152 (%)	n = 139 (%)	n = 202 (%)	n = 116 (%)	n = 225 (%)
<i>2DL1</i>	185 (97.9)	147 (96.7)	136 (97.8)	196 (97.0)	114 (98.3)	219 (97.3)
<i>2DL2</i>	104 (55.0)	89 (58.6)	81 (58.3)	112 (55.4)	68 (58.6)	125 (55.6)
<i>2DL3</i>	165 (87.3)	137 (90.1)	125 (89.9)	177 (87.6)	101 (87.1)	201 (89.3)
<i>2DL4</i>	189 (100)	152 (100)	139 (100)	202 (100)	116 (100)	225 (100)
<i>2DL5</i>	103 (54.5)	78 (53.1)	71 (51.1)	108 (53.5)	61 (52.6)	120 (53.3)
<i>2DP1</i>	186 (98.4)	150 (98.7)	137 (98.6)	199 (98.5)	113 (97.4)	223 (99.1)
<i>2DS1</i>	73 (38.6)	57 (37.5)	51 (36.7)	79 (39.1)	42 (36.2)	88 (39.1)
<i>2DS2</i>	103 (54.5)	81 (53.3)	79 (56.8)	105 (52.0)	67 (57.8)	117 (52.0)
<i>2DS3</i>	50 (26.5)	36 (23.7)	36 (25.8)	50 (25.8)	31 (26.7)	55 (24.4)
<i>2DS4</i>	174 (92.1)	146 (96.1)	126 (90.6)	194 (96.0)	109 (94.0)	211 (93.8)
<i>2DS5</i>	71 (37.6)	57 (37.5)	52 (37.4)	76 (37.6)	43 (37.1)	85 (37.8)
<i>3DL1</i>	177 (93.7)	147 (96.7)	128 (92.1)	196 (97.0)	111 (95.7)	213 (94.7)
<i>3DL2</i>	189 (100)	152 (100)	139 (100)	202 (100)	116 (100)	225 (100)
<i>3DL3</i>	189 (100)	152 (100)	139 (100)	202 (100)	116 (100)	225 (100)
<i>3DP1</i>	189 (100)	152 (100)	139 (100)	202 (100)	116 (100)	225 (100)
<i>3DS1</i>	73 (38.6)	59 (38.8)	53 (38.1)	79 (39.1)	41 (35.3)	91 (40.4)

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

¹ Heterogeneity test – $\chi^2 = 1.26$, $P = 1.00$ and degrees of freedom = 15.

² Heterogeneity test – $\chi^2 = 1.26$, $P = 1.00$ and degrees of freedom = 15.

³ Heterogeneity test – $\chi^2 = 1.53$, $P = 1.00$ and degrees of freedom = 15.

Table 4
KIR alleles frequencies of the present study with three others populations of Paraná state-Brazil by ethnic group.

Genes	Mite-sensitive	Mite-insensitive	Mixed	Caucasoid	Japanese
	n = 211	n = 130	n = 289	n = 164	n = 51
<i>2DL1</i>	98.1	96.2	97.2	97.4	98.0
<i>2DL2</i>	55.0	59.2	47.1	59.4	13.7
<i>2DL3</i>	88.2	89.2	89.3	84.6	98.0
<i>2DL4</i>	100	100	100	100	100
<i>2DL5</i>	54.0	51.5	52.6	51.4	31.4
<i>2DP1</i>	98.6	98.5	96.9	94.7	98.0
<i>2DS1</i>	37.9	38.5	40.8	42.1	33.3
<i>2DS2</i>	54.0	53.8	47.1	59.2	13.7
<i>2DS3</i>	26.1	23.8	26.6	32.5	13.7
<i>2DS4</i>	92.4	96.2	93.8	93.6	94.4
<i>2DS5</i>	37.0	36.9	34.6	31.9	17.7
<i>3DL1</i>	93.8	96.9	94.1	92.7	94.1
<i>3DL2</i>	100	100	100	100	100
<i>3DL3</i>	100	100	100	100	100
<i>3DP1</i>	100	100	nt	nt	nt
<i>3DS1</i>	38.4	39.2	39.1	37.3	29.4

Nt = not tested.

between our study and the other populations except the frequencies of KIR *2DL2*, *2DL5*, *2DS2*, *2DS3*, *2DS5* and *3DS1* in the Japanese population. The distribution of these allelic frequencies does not differ from the other populations observed (allelefrequencies.net). Therefore, Japanese descendants were excluded from our sample.

In Table 5, frequencies of AA and Bx haplotypes were compared with the same populations as above. Japanese offspring presented a higher frequency for AA haplotype (60%), while Caucasoids for

Table 5
Haplotypes frequencies AA and Bx in our studies and others populations by ethnic group.

	Mite-sensitive n = 211	Mite-insensitive n = 130	Caucasoid* n = 164	Mixed** n = 289	Japanese*** n = 51
AA	29.9%	27.7%	28.8%	33.2%	60.0%
Bx	70.1%	72.3%	71.2%	66.0%	40.0%

* Caucasoid from Paraná, Brazil [38].

** Mixed from Paraná, Brazil [37].

*** Japanese from Paraná, Brazil [39].

Haplotype Bx (71.2%). No significant difference was found in the AA and Bx frequencies between the sensitive and non-sensitive individuals and the caustic and admixture populations.

No association was found between the frequencies of KIR genes and their ligands (HLA-C1, HLA-C2, HLA-Cw4, HLA-Bw4, and HLA-A3/A11) for any mite species (*D. farinae*, *D. pteronyssinus*, and *Blomia tropicalis*) or controls.

4. Discussion

It is well established that KIRs play an essential role in the regulation of NK cell activity, in the activation/inhibition of response to class I HLA ligands and as a regulator of the course of viral infections and tumor transformation [40]. Numerous studies have demonstrated the involvement of KIR genes in the pathogenesis of several autoimmune diseases, such as rheumatoid arthritis [41], ankylosing spondylitis [42], psoriasis [43] and systemic lupus erythematosus [44]. However, to the best of our knowledge, this is the first study to demonstrate a relationship between mite hypersensitivity and KIR genes. From this relationship, we did not find significant statistical difference among the studied groups, however, we believe that even though we did not find such differences, these data open space for the discussion of this new relationship, not yet investigated by other researchers.

Regarding the data analysis, of complex genes such as HLA and KIR, Hollenbach et al. [36], consider that there are some limitations that permeate this data. Among the specific questions for KIR data in disease studies are: the treatment of presence/absence or gene-content typing data; the incorporation of HLA interaction effect; the controlling for other HLA associations; the use of estimated haplotypes designations; and the treatment of haplotypic data [36].

Despite a knowledge of the mechanisms of sensitization and triggering of allergic diseases and of several experimental models of allergy, controversies still exist regarding the involvement of regulatory cells and their activated products. The different dust mites allergens have been classified into groups including closely related homologs from various mite species. Until now, 20 different protein families have been officially classified as dust mites allergens (www.iuis.org) and other ones are still under investigation [7]. The allergens that can trigger the activation of the immune response are extremely variable in nature, and most of them contain protease activity, such as group I house dust mites, called cysteine proteases (e.g., Der p 1) [45].

Predictably, some authors have described that the development of Th2 immunity to inhaled house dust mite allergens is associated with different degrees of activity of these proteases [46–49]. These studies opened up new ways of research that revealed the indifferent role of basophils, as dendritic cells alone are sufficient to trigger Th2 responses to inhaled house dust mite allergens/antigens and migrate to lymph nodes [45].

The development of allergic diseases is complex and not fully elucidated, and encompasses genetic and environmental factors. Evidences of tobacco exposure, viral respiratory infections, use of antibiotics, food patterns and exposure to allergens include environmental factors. However, the factors related to heritability, namely, the relationship between the genotypic variance and the phenotypic variance, also show a strong association. The first study to identify the heritability of allergy showed that of a total of 621 sensitized participants, 48.4% had a family history of sensitization to common environmental allergens, compared to only 14.5% of the controls, which totaled 76 individuals not sensitized [50,51]. Years later, the term atopy was given to express hereditary hypersensitivity. Estimates of heritability of atopic diseases are around 95% for asthma, 91% for allergic rhinitis and 84% for atopic dermatitis [51].

Thus, it is possible to affirm that the interaction of genetic variants in the etiology of atopies is well established, in this sense, the studies advance to better understand the pathophysiology of allergic diseases, as well as their biomarkers. Recently, Custovic et al. [52] have demonstrated that different longitudinal patterns in the development of IgE responses to mites during childhood are associated with divergent clinical symptoms, confirming the hypothesis that atopic sensitization can be divided into subtypes, ie, according to the diversity of individual developmental pathways over time, for example, children who have a complete path of sensitization to the mite tend to be at increased risk for asthma (with comorbidities), OR, 7.15; 95% CI, 3.80–13.44, and also a greater chance of airway inflammation [52].

In the meantime, NK cells have also been shown to play a key role in allergic processes by actively participating in antigen-specific immunological suppression and IgE production [53]. Some authors have reported that atopic individuals, such as asthmatic patients, show increased NK cell activity, which decreases after allergen exposure. This can be explained by the fact that these cells are transported through the circulation toward the lungs and lymphoid organs [19].

Niepiekło-Miniewski et al. [54], in a study of atopic dermatitis and KIRs, investigated 240 patients and 570 healthy controls and observed a protective effect of *KIR2DS1* on disease development ($P_{corr} = 0.0158$ and $OR = 0.658$). Atopic dermatitis, due to the immune response to allergic challenge, is considered one of the most common manifestations in mite-sensitive individuals.

The uneven distribution of KIR alleles and the diversity of their haplotypes have been described in several ethnic groups in different populations [55–61].

The AA haplotype group, which consists of the KIR phenotypes *3DL1*, *2DL1*, *2DL3*, *2DS4*, *2DL4*, *3DL2*, *3DL3*, *2DP1*, *3DP1*, was investigated in our study. Comparing the haplotype frequencies of sensitive and non-sensitive individuals with other populations in the state of Paraná, our data present a frequency similar to the Caucasoid and admixture population. Unlike the haplotype frequencies of the Japanese population of Curitiba-PR [39].

The constant search for understanding the distribution of KIR genes in population groups allows us to expand the understanding of their characteristics and determine whether these peculiarities are in some way related to protection/susceptibility to certain diseases. Therefore, we believe these preliminary findings may provide further insight into defining the importance of these genes, and after a deeper analysis of these data, as described, we can more concisely determine the participation (or not) of KIR genes in hypersensitivity to dust mites.

A limitation of the present study is the KIR genotyping method, which determines only the presence/absence of genes; therefore, the

alleles of each gene and the amount of expressed product were not defined. A future perspective from our study would be to conclude that there is no relationship of NK cells to sensitization to mites or conduct more in-depth research at the allele level on genes that regulate the expression of products, on products expressed in soluble forms, on interactions with other HLA-G ligands and on different populations. According Norman et al. [62], the development of methods for assessing the nature and extent of KIR genomic diversity has been limited by the complexity of the region. The widely used methods that exist for typing KIRs focus principally on gene content. In contrast, the methods being used for determining allelic variation are costly, time consuming, and unsuitable for high-throughput studies. The results of the few allele-level population studies of KIRs, however, show that such investigation is likely to be informative. For example, some KIRs are restricted to population groups of specific geographic ancestry. Other KIRs have lost expression but appear common and widely distributed. To extend such studies to other populations, as well as disease cohorts, they have developed a sequencing and bioinformatics method that determines complete KIR and HLA class I genomic diversity. Further advances are still needed in the etiopathogenesis of mite sensitivity to clarify the causes and pathogenic mechanisms underlying this condition.

5. Conclusion

This preliminary study suggests no influence of KIR genes on resistance/susceptibility to sensitization to dust mites. However, we consider that these results are not conclusive enough to reject this hypothesis.

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