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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 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 FceRI 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 α1 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 20 th 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 ηg/μL, with a purity of 1.6–1.9 (OD 260/280).