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Molecular identification of the main allergens present in household dust of asthmatic patients

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ABSTRACT

Introduction: The main aeroallergens present in house dust are the mites, *Dermatophagoidespteronyssinus* (Derp) and *Dermatophagoidesfarinae* (Derf), and cockroaches, *Periplanetaamericana* (Pera). Objective: This work aims to genetically identify the allergens Derp, Derf, and Pera in household dust of asthmatic patients. Materials and methods: 29 patients, aged between 3 and 17 years, were classified as asthmatic or non-asthmatic according to the International Study of Asthma and Allergies in Childhood (ISAAC). Subjectscompleted a complementary questionnaire and skin hypersensitivity tests were performed. House dust was collected from these patients, filtered, and then DNA was extracted. Polymerase chain reactions were performed to identify Derp, Derf, and Pera in the samples. Results: There was an association between Pera sensitization and onset of asthma. There was also an association between the presence of Derp in the home of asthmatic patients and the worsening of symptoms, such as wheezing in the chest and allergic rhinitis. An association between the presence of Derf in house dust of asthmatic patients and the symptoms of allergic rhinitis was found. These data suggest that cockroach sensitization is a predominant factor in asthmatic children and the presence of mite allergens contributes to the worsening of asthma symptoms.

Keywords: Asthma; Dust mite; Cockroaches

1 INTRODUCTION

Asthma is characterized as a multifactorial disease, with complex interactions between environmental and genetic factors (POMÉS; ARRUDA, 2014). It is estimated that 334 million people worldwide have asthma, and that the prevalence of this disease has increased in the last few decades, mainly among children (TO *et al.*, 2012). In Brazil, asthma

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is a highly prevalent disease, with an average of 23.3% of children and 22.7% of adolescents being impacted and, thus, asthma should be considered a Public Health problem (SOLE *et al*. 2014).

The disease is characterized by recurrent episodes of airway obstruction, where reversal may occur spontaneously or after the use of medication, and it is generally associated with bronchial hyperresponsiveness and chronic inflammation of the airways (BERTELSEN *et al*., 2017). Clinical manifestations consist of recurrent episodes of wheezing, dyspnea, chest tightness, and coughing, which are typically aggravated during the night or early morning (JUST; BOURGOIN-HECK; AMAT, 2017). Symptoms may disappear for a few months; however, exacerbations can be severe (GIBSON, MCDONALD, 2017).

The pathogenesis of asthma is related to type I hypersensitivity with involvement of T helper 2 (Th2) $CD4^+$ T cells and type 2 innate lymphoid cells (ILC2). These cells produce cytokines such as interleukin-13 (IL-13), IL-4 and IL-5, which play a key role in the activation of eosinophils and in the activation of B lymphocytes leading to Immunoglobulin E (IgE) production. The Fc portion of IgE antibodies binds with high affinity to receptors present on mast cells, which activate and degranulate in the presence of allergens, triggering allergic and inflammatory processes in the lung (WALSH, 2017).

Inhalant allergens, which include house dust mites (*Dermatophagoidespteronyssinus* (Derp), *Dermatophagoides farina* (Derf) and *Blomia tropicalis* (Blot)), cockroaches (*Periplanetaamericana* (Pera) and *Blattellagermanica* (Blag)), domestic animals (dog and cat) and fungi, have been identified as the major allergens responsible for respiratory allergies and asthma (GIBSON, MCDONALD, 2017, LAWSON *et al*., 2017).

Mites are microscopic arthropods and taxonomically related to ticks, spiders, and scorpions (SOLARZ; BREWCZYNSKI, 1999). Mites are abundantly present in domestic dust and considered to be triggers of allergic asthma worldwide (CHARPIN, 2012). Some domestic dust mites have a cosmopolitan distribution, such as Derp. Derf is the second most abundant species globally, although it is more abundant in North America than in Europe. Blotis more commonly found in tropical and subtropical regions. Several other

species are present in a regional distribution, but all species have the potential to be allergenic (BERGMANN; RAULFFS; SANDER, 2014). Biotic and abiotic factors can also influence the distribution of Acarina fauna in re all over the world.

The understanding of allergy to cockroaches began in 1964 with Bernton and Brown, who detected a positive skin test to cockroach extracts. In 1979, Kang *et al*. demonstrated early, late, and double bronchoconstriction following inhalation of a cockroach antigen by asthmatic and allergic patients, thus making a relationship between a cockroach allergy and asthma. Despite the existence of thousands of species of cockroaches, only 25 have adapted to live in a domestic habitat, such as the German cockroach (Blag), commonly found in cold and dry climates, and the American cockroach (Pera), which is predominately found in hot and humid areas (POMÉS, ARRUDA, 2014).

Local climatic conditions are fundamental for the development of mites and determine which species is predominant in each region. A high population density associated with adequate altitudes and climatic conditions is an environment conducive to the development and reproduction of mites (HENSZEL, 2006).

The objective of this study was to carry out the genetic identification of Derp and Derf mite allergens and Pera cockroach allergens in the household dust of asthmatic patients from Northeast Brazil.

2 MATERIALS AND METHODS

2.1 Patients

For this study, 29 patients, aged 3 to 17 years, were classified as asthmatic or non-asthmatic. Asthma was clinically diagnosed according to the International Study of Asthma and Allergies in Childhood (ISAAC) questionnaire. These criteria take into account presence of one or more symptoms, such as dyspnea, chronic cough, wheezing, chest tightness or chest discomfort, occurring at night or in the early hours of the morning; spontaneous improvement or the use of specific medications for asthma (bronco dilators, steroid anti-inflammatories); and three or more episodes of wheezing in the last year. In addition, a supplementary questionnaire was used to assess seasonal variability of symptoms, positive

family history for asthma or atopy, environmental factors, and excluded alternative diagnoses. These diagnoses were performed by specialists in the field. Patients with chronic diagnosis of respiratory diseases were not included in this study.

The parents or legal guardians of the children involved in the study gave their informed consent, and all procedures described herein do not exceed the minimum risk limits in accordance with the Helsinki Convention for Human Research and National Health Council regulations. The project is presented under protocol number 58737916.3.0000.5084.1.1.1.

2.2 Immediate hypersensitivity skin test

Atopic sensitization was evaluated through the immediate hypersensitivity test (pricktest), performed with Derp, Derf and Pera allergens. The negative control used was a buffered saline solution, and the positive control was histamine. All applications were made in the forearm region, and after 15 minutes, the reading was done with the aid of a ruler. The reaction was considered positive when a papule was formed with a size equal to or greater than 3 mm in diameter.

2.3 Collection, filtering and homogenization of household dust samples

The dust samples were collected from the surface of the bed and underneath the bed. A hand-held vacuum cleaner (Consul, Brazil), equipped with a cotton and polyester trap fitted at the end of the aspirator tube, was used with a sterile plastic bag to collect the dust samples. Each square meter of surface was aspirated for 8 minutes. At the end of each collection, the cleaner tip was cleaned with water and alcohol. The samples were stored in a freezer at -20ºC. The dust particles were separated from the plastic bag under laminar flow, using a sterile curette. They were then filtered through a sterile sieve with pore sizes of 300 to 355 µm in diameter (EndecottsLtd, London, UK), to remove larger fibers and particles and obtain a homogeneous powder. The samples were then weighed. The intention was to obtain particles of 6 to 300 µm diameter. The dust extracts were stored in sterile polypropylene tapered tubes (BD System, Franklin Lakes, NJ, USA).

2.4 Molecular identification of mites and cockroaches

2.4.1 DNA extraction

DNA extraction was performed using the Genomic DNA Purification Kit (Promega). After extraction, the concentration and degree of purity of each sample was determined by the NanoDrop 1000 apparatus (ThermoScientific, DE, USA).

2.4.2 PCR (Polymerase Chain Reaction)

The PCR reaction was done with a final volume of 20µl using 0.2ml microtubes and the corresponding primers for PCR amplification. Each reaction contained 10µl of PCR Master Mix (Promega, Brazil), 0.4µL of each primer (table 1), 7.2µL of nuclease free water and 2µl of sample DNA. The PCR program consisted of an initial denaturation at 95°C for 5 minutes, followed by 35 cycles of denaturation at 94°C, hybridization at 60°C and extension at 72°C for 30 seconds each. After the last cycle, a final extension step was performed at 72°C for 10 minutes (Thermofisher, Brazil). Aliquots of 5µL of the PCR product were analyzed by gel electrophoresis with 2% agarose, and Syber safe (Invitrogen, Brazil) was added for visualization of the DNA in the UV Transilluminator. A 100-bp DNA marker (Invitrogen, Brazil) was used to identify the size of the fragment.

Table 1 - Primers used in the PCR reaction

Species	Forward Primer 5'-3'	Reverse Primer 5'-3'	Base Pairs
Derp	CTCAGGTTGTTACTTGTGGG	CATCCATCTAGCCCGAGGAA	209
Derf	CGTAGGTTGAACATAGAGAAC	CAATACACCCATTAACACCG	705
Pera	AGTTATGGTTCCTTAGATGGTG	AGCACTCTAATTTGTTCAAAGTA	749

2.4.3 Statistical analysis

Statistical analysis was performed using the IBM-SPSS program. The data were evaluated using the Chi-square test of independence or Fisher's exact test. The significance level adopted was 5%, that is, the probability of $p<0.05$ is capable of revealing statistically significant differences between the data from the different groups.

3 RESULTS

For this study, 29 patients, aged between 3 and 17 years, were selected, of whom 18 patients were classified as non-asthmatics and 10 were classified as asthmatic. The "prick" test was performed for patient sensitization analyses. It was observed that four (40%) of the asthmatic patients were sensitized by the Derp allergen, three (30%) by Derf, and four (40%) by Pera (Table 2). A significant association was observed between patients were sensitized to Pera allergens and asthma (Table 2, p = 0.040).

Table 2 - Sensitization to Derp, Derf, and Blot allergens for non-asthmatic and asthmatic patients

Sensitized	Non-asthmatic	Asthmatic	p
Derp			
No	8 (44.4%)	6 (60%)	0.690
Yes	10 (55.6%)	4 (40%)	
Derf			
No	9 (50%)	7 (70%)	0.430
Yes	9 (50%)	3 (30%)	
Pera			
No	17 (94.4%)	6 (60%)	0.040
Yes	1 (5.6%)	4 (40%)	

After sensitivity analyses, household dust was collected from non-asthmatic and asthmatic patients, and molecular identification of Derp, Derf, and Pera allergens in the samples was performed. The results showed that there was no association between the presence of allergens in house dust and the onset of asthma (Table 3).

Table 3 - Identification of Derp, Derf, and Pera allergens in household dust of non-asthmatic and asthmatic patients

Variable	Non-asthmatic	Asthmatic	p
Total	18 (100%)	10 (100%)	
Dust mite Derp			
Absent	2 (11.1%)	0 (0.00%)	0.520
Present	16 (88.9%)	10 (100%)	
Dust mite Derf			
Absent	13 (72.2%)	8 (80%)	1.000
Present	5 (27.8%)	2 (20%)	
Dust mite Pera			
Absent	13 (72.2%)	9 (90%)	0.370
Present	5 (27.8%)	1 (10%)	

In addition, we analyzed the association between sensitization and the presence of dust mites, and the results showed that there was no association between the presence of allergens in the house dust and sensitization to them (Table 4).

Table 4 - House dust allergens in non-sensitized and sensitized patients

Dust mite	Sensitized		p
Derp	**Non-Sensitized**	**Derp sensitized**	
Absent	2 (14.3%)	0 (0.0%)	0.480
Present	12 (85.7%)	14 (100%)	
Derf	**Non-Sensitized**	**Derf sensitized**	
Absent	11 (68.8%)	10 (83.3%)	0.660
Present	5 (31.3%)	2 (16.7%)	
Pera	**Non-Sensitized**	**Pera sensitized**	
Absent	18 (78.3%)	4 (80%)	1.000
Present	5 (21.7%)	1 (20%)	

Statistical analysis of the three variables was performed to determine if the presence of Derp, Derf, and Pera in the dust of the homes of asthmatic and non-asthmatic patients was associated with the onset of symptoms such as wheezing and allergic rhinitis. The data showed that the presence of the Derp allergen in household dust was associated with chest wheezing (table 5, p = 0.001) and allergic rhinitis (table 5, p = 0.004) in asthmatic patients. The presence of the Derfallergen in household dust was associated with the symptoms of allergic rhinitis in asthmatic patients.

Table 5 - Analysis of association between the presence of allergens in household dust of non-asthmatic and asthmatic patients and symptoms of wheezing and allergic rhinitis

	Presence of Dust Mite - Derp		
	Non-asthmatic	**Asthmatic**	**p**
Wheezing			
No	15 (93.8%)	3 (30%)	0.001
Yes	1 (6.3%)	7 (70%)	
Rhinitis			
No	12 (75%)	1 (10%)	0.004
Yes	4 (25%)	9 (90%)	
Wheezing			
No	5 (100%)	1 (50%)	0.286
Yes	0 (0 %)	1 (50%)	
Rhinitis			
No	5 (100%)	0 (0%)	0.048
Yes	0 (0 %)	2 (100%)	
	Presence of Dust Mite - Pera		
Wheezing			
No	5 (100%)	0 (0%)	0.167
Yes	0 (0 %)	1 (100%)	
Rhinitis			
No	2 (40%)	0 (0%)	1
Yes	3 (60%)	1 (100%)	

4 DISCUSSION

Sensitization to an aeroallergen depends on the relationship between genetic susceptibility and exposure to the allergens. Each individual has a genetic inheritance that impacts the probability of gene activation, just as each population has its environmental conditions that can activate those genes, which can lead to an increased susceptibility of disease. It is well known that mite and cockroach allergens are fundamental environmental factors for sensitization and the triggering of allergy symptoms and asthma. This work shows the importance of evaluating the environmental factors that may be involved in the onset of asthma.

Previous literature has shown the relationship of sensitization to allergens and the triggering of allergic diseases and asthma. In this work, the results showed that sensitization to Pera allergens was associated with asthma symptoms. This data supports observations from Poland where approximately 25% of asthmatic children are sensitized to cockroach allergens (PAGAN, 2012). In the United States, it has been found that approximately 60% to 80% of children living in urban areas are sensitized to cockroaches (GUILLEMINAUL, L., 2017). Additionally, a study in Taiwan showed that 58% of asthmatic children were sensitized to the Pera allergen (DUTRA, 2018).

Cockroach infestations in urban environments are more commonly found in countries that are hot and humid, which applies to the state of Maranhão. Environmental interventions, such as applying cockroach pesticides in homes with asthmatic children, were associated with a reduction of asthmatic crises in these children (FAZLOLLAHI, MR, 2019). Another study showed that the strategic interventions of insecticide baits have low toxicity, low cost and resulted in an elimination of cockroaches for 12 months, which was related to a reduction of asthmatic crises (RABITO, 2017).

Other allergens that have been described as factors for the onset of asthma are house dust mites, which are the largest source of allergens in several countries, such as Europe, Asia, South America, the United States, New Zealand, Australia, and Africa (THOMAS, 2010). More than 10% of the world's population and 90% of patients with allergic asthma are sensitive to mites (CHAN SL, *et al*., 2008). Among mite allergens, *Dermatophagoides pteronyssinus* (Derp) and *Dermatophagoides farinae* (Derf) are the most prevalent species and are found in multiple locations around theglobe. *Blomia tropicalis* (Blot) is typically only found in tropical countries (DUTRA, 2016).

According to previous studies performed in Brazil, Derp predominates in household dust from the cities, São Paulo and Salvador, whereas Derf was the predominant species present in household dust from São Paulo and Belo Horizonte. In Uberlândia and Uberaba, both species were found (RIZZO *et al*., 1997). The results from our study showed that all three mites were present in the houses of both asthmatic and non-asthmatic children. The presence of these mites in household dust was not associated with asthma triggers or sensitization to them. However, the presence of Derp in the house dust of asthmatic patients was associated with symptoms of wheezing and allergic rhinitis. The presence of

Derf in the house dust of asthmatic children's homes was associated with the symptoms of allergic rhinitis. The same results were not observed with the Pera allergen. Previous work has shown that the presence of dust mites in household dust can lead to the development of allergic rhinitis and an increase in asthmatic attacks (BAIZ, N, 2019).

Taken together, this data suggests that the Peraallergen is associated with the onset of asthma in children, and that the presence of Derp and Derf does not contribute to the onset of asthma, but can worsen the asthmatic crises by increasing wheezing and rhinitis. Asthma and allergic diseases are multifactorial and highly complex with both genetic and environmental influences. Understanding the role that these environmental factors play is crucialinimproving quality of life in asthmatic patients.

5 CONCLUSIONS

There is an association between sensitization to the Pera allergen and the presence of asthma symptoms. There was no association between the presence of allergens in house dust and the development of asthma and allergen sensitization; however, there was an association between the presence of Derp in the house dust of asthmatic patients and the symptoms, including wheezing and allergic rhinitis. The presence of allergens in the dust of homes of asthmatic patients were associated with the symptoms of allergic rhinitis. This data suggests that cockroach sensitization is a predominant factor for development of asthma in children, and that the presence of mite allergens is important for the worsening of asthma symptoms.

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