IgE sensitization to Blo t 21 and Blo t 5 is associated with asthma in the tropics: a
case-control study

Short title: Blo t 5 and Blo t 21 are associated with asthma

Zakzuk J\textsuperscript{1}, Donado K\textsuperscript{1}, Mondol E\textsuperscript{1}, Marrugo V\textsuperscript{1}, Regino R\textsuperscript{1}, López JF\textsuperscript{1}, Hernández K\textsuperscript{1},
Mercado D\textsuperscript{1}, Dennis R\textsuperscript{2}, Puerta L\textsuperscript{1}, Caraballo L\textsuperscript{1}

\textsuperscript{1}Institute for Immunological Research, University of Cartagena, Cartagena, Colombia
\textsuperscript{2}Fundación Cardioinfantil, Bogotá, Colombia

Corresponding author:
Luis Caraballo, MD, PhD
Universidad de Cartagena. Campus de la Salud. Cra. 50 #24-120
lcaraballog@unicartagena.edu.co

This article has been accepted for publication and undergone full peer review but has not been
through the copyediting, typesetting, pagination and proofreading process, which may lead to
differences between this version and the Version of Record. Please cite this article as doi:
10.18176/jiaci.0892
Abstract

**Background:** *Blomia tropicalis* sensitization is associated with asthma in different tropical and sub-tropical countries; however, information about the specific molecular components associated with this disease is scarce.

**Objective:** Using molecular diagnosis, we sought to identify *B. tropicalis* allergens associated with asthma in Colombia.

**Methods:** Specific IgE (sIgE) to eight *B. tropicalis* recombinant allergens (Blo t 2/5/7/8/10/12/13 and 21) was determined using an in-house developed ELISA system in asthmatic patients (n=272) and control subjects (n=298) recruited in a national prevalence-study performed in Colombian cities (Barranquilla, Bogotá, Medellín, Cali and San Andrés). Sample study included children and adults (mean age: 28±SD 17 years old). Cross-reactivity between Blo t 5 and Blo t 21 was evaluated by ELISA-inhibition.

**Results:** Sensitization to Blo t 21 (aOR: 1.9; 95% CI: 1.2 – 2.9) and Blo t 5 (aOR: 1.6; 95%CI: 1.1 – 2.5), but not Blo t 2, was associated with asthma. sIgE levels to Blo t 21 and to Blo t 5 were significantly higher in the disease group. Cross-reactivity between Blo t 21 and Blo t 5 is on average moderate; however, individual analysis indicates that may be high (>50%) in some cases.

**Conclusions:** Although Blo t 5 and Blo t 21 has been described as common sensitizers, this is the first report of their association with asthma. Both components should be included in molecular panels for allergy diagnosis in the tropics.
Key words: Asthma. Molecular diagnosis. Component-resolved diagnostics. Allergy. 
Case-control study. Precision medicine. 

Resumen 

Antecedentes: la sensibilización a Blomia tropicalis está asociada con asma en diferentes países tropicales y subtropicales; sin embargo, la información sobre los componentes moleculares específicos asociados con esta enfermedad es escasa. Objetivo: Mediante diagnóstico molecular se buscó identificar alérgenos de B. tropicalis asociados al asma en Colombia. 

Métodos: Se determinó la IgE específica (sIgE) a ocho alérgenos recombinantes de B. tropicalis (Blo t 2/5/7/8/10/12/13 y 21) usando un sistema ELISA desarrollado internamente en pacientes asmáticos (n=272 ) y sujetos control (n=298) reclutados en un estudio de prevalencia nacional realizado en ciudades colombianas (Barranquilla, Bogotá, Medellín, Cali y San Andrés). La muestra del estudio incluyó a niños y adultos (edad media: 28±DE 17 años). La reactividad cruzada entre Blo t 5 y Blo t 21 se evaluó mediante ELISA inhibición. 

Resultados: La sensibilización a Blo t 21 (aOR: 1,9; IC 95 %: 1,2 a 2,9) y Blo t 5 (aOR: 1,6; IC 95 %: 1,1 a 2,5) se asoció con asma, pero no a Blo t 2. Los niveles de sIgE para Blo t 21 y para Blo t 5 fueron significativamente más altos en el grupo de enfermedad. La reactividad cruzada entre Blo t 21 y Blo t 5 es en promedio moderada; sin embargo, el análisis individual indica que puede ser alto (>50%) en algunos casos.
**Conclusiones:** Aunque Blo t 5 y Blo t 21 han sido descritos como sensibilizantes comunes, este es el primer estudio de su asociación con el asma. Ambos componentes deben incluirse en paneles moleculares para el diagnóstico de alergias en los trópicos.

**Palabras clave**

Introduction

*Blomia tropicalis* is one of the most important causes of sensitization in the Tropics [1]. Sensitization to this house dust mite (HDM) is associated with asthma acute exacerbations in case-control studies [2-4], but few studies have identified which of its allergens are specifically related with disease presentation. Precision medicine in allergy is based on the identification of clinically relevant allergens for several applications such as the development of molecular reagents for allergen standardization and formulation, measurement of allergen exposure, and designing new strategies for allergen immunotherapy such as hypo-allergenic vaccines [5-7].

Early and strong IgE responses to *Blomia tropicalis* are commonly detected in people living in tropical settings (8). Previous investigations in the "Risk Factors for Asthma and Allergy in the Tropics" (FRAAT) cohort also support that children may be early sensitized to molecular components without any disease manifestation [8]. Then, it is important to define the *Blomia tropicalis* allergens that are associated with allergic diseases.

Fourteen *B. tropicalis* allergens have been officially recognized by the IUIS [9]. Blo t 5 [10] and Blo t 21 are phylogenetically related allergens [11,12] and have been recognized as most common sensitizers from this HDM in some populations [13]. Several epidemiological surveys have shown that other IgE binding molecules from *B. tropicalis* are frequent sensitizers [14-17]. Blo t 2, for example, has low to moderate cross-reactivity with Group 2 from *Dermatophagoides spp* [14], and it has been proposed as clinically relevant in Brazil; in combination with Blo t 5 and Blo t 21, it confers higher sensitivity to diagnose *B. tropicalis*
sensitization using molecular panels [15]. Blo t 7 sensitized more than >50% of Singaporean allergic children [16] and Blo t 13, as well as other fatty acid binding allergens, may induce the activation of biological pathways that promotes airway inflammation [17]. Blo t 12, a species-specific allergen of *B. tropicalis* with chitin binding activity [18] and Blo t 8, a mu-like glutathione transferases, have been described as IgE binding proteins with ability to induce mast cell degranulation responses [19].

A group of investigators working on indoor allergens has suggested that the association of sensitization with disease presentation, as well as other factors, are important criteria to define allergens of clinical relevance for diagnosis and treatment [20], which is currently done mainly based on the frequency of sensitization [21]. In this study, by using a panel of 8 allergens of *B. tropicalis*, under an exploratory analysis in a nested case-control study [22], we sought to evaluate the association of IgE sensitization to specific components with asthma presentation in patients recruited in a tropical environment.

**Methods**

*Study design and setting*

From a nested case-control study within a population-based survey performed in 2009-2010, we randomly selected a sub-sample of 272 cases and 298 control subjects with the aim to compare the prevalence of sensitization to eight allergens of *Blomia tropicalis*. A description of the ancillary study is published elsewhere [22]. Participants between 1 and 59 years old were included in the study. People in acute care hospitals or institutions for the chronically
ill or for the disabled at the time of the study were excluded as well as people with an altered mental state, dementia, or mentally challenged. Serum samples were collected from participants and stored at -80°C. In addition, we randomly selected a sub-sample of cases and controls from each city. This protocol was approved by the Ethics Committee of the University of Cartagena (Acta 10-05-2018).

Definition of asthma was done after the application of a previously validated ISAAC-based questionnaire [23,24]. A “case” was defined as a participant reporting current or past asthma symptoms. A “control” was defined as any subject who answered “no” to questions indicative of having symptoms of asthma, allergic rhinitis, or atopic eczema in the last year; and without any medical antecedent of allergic diseases [22]. Descriptive features of study participants are shown in Table 1. Sample study included children and adults (mean age: 28±SD 17 years old). Cases and controls had no differences in age, but male sex was significant more frequent in controls (32.7 vs 41.6%)

Study location
Colombia is a South American country; due to its location in the intertropical zone and the presence of the Andeans Mountains, it has a large variety of weathers and ecosystems. This study was performed in five main cities of the country: Bogotá, Barranquilla, Cali, Medellín and San Andrés. Barranquilla and San Andrés Islands, in the North region, are mostly at the sea level and share similar hot (~28-29°C) and humid weather conditions. Medellín (~22°C), Cali (~24°C), and Bogotá (~13°C) are located on different branches of the Andes at 1,480,
1,018 and 2,582 meters of altitude, respectively. These cities tend to have constant temperature across the year, with lower temperatures in the higher cities.

Recombinant allergens

Blo t 5, Blo t 8 and Blo t 21 were isolated from a cDNA library built with mites collected in house dust obtained in Cartagena, Colombia and directly cloned from the PCR reaction into a pET100 vector. Blo t 12 and Blo t 13 were isolated from the same *B. tropicalis* cDNA library [25] and their sequences were codon-optimized and cloned into pET45b+ [6,18]. Codon-optimized sequences of Blo t 2 (accession number: A1KXI4) and Blo t 10 (accession number ABU97466.1) were synthesized and cloned into pET-45b+ vector by Genscript (Piscataway, N.J., USA). Production of Blo t 5 [26], Blo t 8 [27], Blo t 10 [28], Blo t 12 [18] and Blo t 13 [29] have been published elsewhere. Production of the rest of allergens is described here. Competent *E. coli* BL21 (DE3) cells (for Blo t 7 and Blo t 21) and *E. coli* Origami (DE3) cells (for Blo t 2) were transformed with the respective recombinant plasmids by electroporation (MicroPulser™ Bio-Rad, Hercules, CA., USA) and selected on Luria Broth-agar plates containing 100 µg/mL ampicillin (LBA). Protein expression was induced in the logarithmic phase with 1 mM IPTG for 4-5 hours at 37°C in most cases, except Blo t 7, whose production was induced with 0.5 mM IPTG at 25°C for 24 hours. lo t Bacterial pellets of allergen cultures were recovered and resuspended in 20 mM NaH$_2$PO$_4$ 0.3 M NaCl (NBB pH 8.0). Lysis and purification of each recombinant protein was standardized independently, but in general, all molecules were lysed by sonication (Sonic dismembrator FB-705, Fisher scientific, Waltham, MA., USA) and with
lysozyme (100 ug/mL. Proteins were purified by affinity chromatography using a Ni2+ NTA resin (Qiagen, Cat 30230, Hilden, Germany) and 250 mM imidazole NBB pH 8.0 as elution buffer and further lyophilized and stored at -20°C in the “Silvia Jimenez” Recombinant Allergens’ bank.

Assessment of sensitization

Specific IgE antibody levels to the house dust mites D. pteronyssinus and B. tropicalis were measured using the ImmunoCAP system as previously described [21]. Specific serum IgE levels to the 8 recombinant allergens were detected in duplicate using a multiplex indirect ELISA- based platform. Each allergen (0.25 µg) was coated on carbonate/bicarbonate buffer (pH 9.2) by ON incubation. After five washes with 0.1% Tween 20 PBS, wells were blocked with 1% PBS bovine serum albumin with 0.02% sodium azide. After washing (4X) serum (1:5) was added and incubated ON at room temperature (RT) in a humid chamber; then washed 5X and incubated for 2 hours with anti-human IgE ε- chain specific - alkaline phosphatase conjugate (Sigma, St. Louis, USA), diluted 1:1000 in buffer Tris 50mM 1% BSA MgCl2 1mM with 0.02% sodium azide (pH 8) and developed with p- nitrophenyl phosphate substrate (1 mg/ml; Sigma, St. Louis, USA) diluted in 10% diethanolamine, 0.5 mM MgCl2 (pH 9.8). The reaction was incubated in the dark, stopped after 60 minutes with 50 µl of 3N NaOH and absorbance was measured at 405 nm in a spectrophotometer (Multiskan GO; Thermo Scientific, Vantaa, Finland). A negative control serum for all the evaluated allergens and B. tropicalis extract (Lot #28, Institute for Immunological Research, University of Cartagena) was used in each experiment, while PBS was used as a control for
non-specific binding of anti-human IgE alkaline phosphatase conjugate. As for the positive control, a curve with two-fold serial dilutions (from 1:5 to 1:80) was made using a serum with known IgE reactivity against *B. tropicalis* extract. These dilutions were made for each experiment in duplicate, using wells coated with 0.5 µg *B. tropicalis* extract diluted in buffer carbonate/bicarbonate (pH 9.2). Cutoffs values to define positive or negative IgE responses to the recombinant allergens were calculated as the mean optical density (OD) of 11 negative, non-mite/sensitized controls + 3 SD. The cutoff value to define sensitization was 0.09 OD units.

Cross-inhibition assays

To study cross-reactivity between Blo t 5 and Blo t 21, inhibition ELISA was carried out according to a similar protocol described earlier [30]. Serum pools of double-sensitized patients were absorbed with 10-fold serial dilutions of allergens (from 1 ng/mL to 100 mg/mL). A description of serum used in the pools is presented in Supplementary Table 1. We also performed end-point inhibition ELISAs using five individual sera of patients sensitized to both allergens that were incubated with 0.1 mg/mL of Blo t 21 or Blo t 5. Results are shown as percentage of inhibition, calculated as follows = OD without inhibitor - OD with inhibitor/OD without inhibitor * 100. Bovine serum albumin (BSA) was used as non-related inhibitor.
Statistical analysis

Univariate and multivariate binary logistic regression models were used to evaluate the association of asthma presentation as outcome with sensitization to each *B. tropicalis* molecular component. *A priori* adjusting variables were age, gender, socioeconomic stratum, and city of residence. Crude (OR) and adjusted odd ratios (aOR), and their respective 95% confidence intervals (95% CI) are reported. An association was considered significant when p-value was <0.05. Due to data distribution, non-parametric tests were chosen for analyses on specific IgE values. Allergen-specific IgE was compared between groups using the Mann-Whitney U test. Correlation of log transformed IgE values was analyzed by Pearson test. Statistical analyses were carried out using SPSS version 25 (SPSS Chicago, IL, USA).

Regarding data visualization, upset plots were generated with ComplexUpset package (version 1.3.3) in R version 4.1.1 (2021-08-10) to count the number of subjects with a positive IgE response to one or multiple allergens (intersections). Bar and strip plots depicting the distribution of sensitizations among groups were generated with seaborn version 0.11.2. in Python 3.9 (64-bit).

RESULTS

*IgE response to Blo t 21 and to Blo t 5 is associated with asthma*

Blo t 2 was the most common sensitizer in both patients and controls (Figure 2), with no significant differences in frequency between groups (Figure 1). In contrast, Blo t 21 and Blo
t 5 sensitization was significantly greater in the disease group (Table 2). After adjusting by potential confounders and, in independent models, sensitization to Blo t 21 (aOR: 1.83, 95% CI: 1.18 – 2.83, p = 0.007) and to Blo t 5 (aOR: 1.61, 95% CI: 1.05 – 2.47, p = 0.03) were significantly associated with asthma (Table 3). Although sex was associated with disease presentation in both models, it did not show a modifier effect on the detected associations (Table 3). The strength of the IgE response to B. tropicalis allergens was also analyzed regarding disease status (Figure 2, Table 4). Specific IgE levels to Blo t 5 (p=0.014) and Blo t 21 (p=0.029) were also significantly higher in patients than in controls. For the rest of allergens, there were not differences among groups (Table 4).

Sensitization to any of these two allergens (Blo t 5 or 21) was observed in 27.6% of asthmatic patients (n=75) and 18.8% of controls (n=56). In the adjusted model, the strength of association of sensitization to any of the two components was not higher (aOR: 1.61, 95% CI: 1.08 – 2.41, p = 0.02) than for Blo t 21 alone.

The degree of cross-reactivity between Blo t 21 and Blo t 5 is variable among individuals

Most patients sensitized to Blo t 21 were also sensitized to Blo t 5 (86.1%). In Blo t 5 sensitized patients, co-sensitization to Blo t 21 was also common (80.3%). As shown in the correlation matrix (Figure 3A), log specific IgE to Blo t 5 and Blo t 21 showed the highest value among all possible component pairs (r= 0.87, p<0.001, Figure 3B). By ELISA inhibition, using a serum pool positive to both allergens, a moderate level of IgE inhibition of Blo t 21 to Blo t 5 binding (54%) was observed at the highest inhibitor concentration. In
the contrary, Blo t 5 inhibited in a lower grade (29%) the IgE binding to Blo t 21 (Figure 3C). However, by analyzing individual sera we could observe that Blo t 5 can also inhibit in a great level to Blo t 21, with values ranging from 2 to 81% (Figure 3D).

**Asthmatics had a higher response rate to the molecular panel**

In asthmatics, 108 out of 272 patients were positive at least for one allergen (39.7%); meanwhile, 94 out of 298 subjects (31.5%) were sensitized in the control group (OR: 1.60, 95% CI: 1.13-2.26; p = 0.008). Sensitization frequency detected with the *B. tropicalis* panel was slightly higher than that found with the complete extract in the asthma group (36.8%), and ~12% higher in the control group (19.1%). Sensitization to any allergen was also associated with asthma even after adjustment by age, sex, city of residence and SES (aOR: 1.55, 95% CI: 1.09-2.22; p = 0.014).

We also explored other patterns of sensitizations in cases and controls by analyzing intersection sets. As observed in Figure 4, Blo t 2 monosensitization was the most frequent condition, both, in cases (16/108 sensitized individuals, 14.8%) and controls (21/94, 22.3%). In both groups, the second most common sensitization pattern was a positive IgE result to the eight components. Blo t 2/Blo t 5/Blo t 21 sensitization was the third most common frequent combination (followed by Blo t 5/Blo t 21) among cases, but not in controls.

There were significant differences in the rates of sensitization to molecular components among cities. Barranquilla and San Andrés (two cities at the sea level) showed the highest rates of sensitization to Blo t 2, Blo t 5 and Blo t 21 (Supplementary Figure 1). However,
inclusion of city or residence in the multivariate logistic regression models did not show a modifier effect on disease presentation (Table 3).

**DISCUSSION**

In this study, we report the associations between sensitization to Blo t 21 and to Blo t 5 with asthma in a tropical country. This is the most complete evaluation of a molecular repertoire of *B. tropicalis* allergens using a case-control design. Most of the allergens included have at least skin-prick test (SPT) or basophil activation test (BAT) showing allergenic activity [12,19,20,31-34]. Among the eight allergens evaluated, only two showed significant associations with asthma; in addition, the strength of the IgE response was also significantly greater among patients, suggesting that these allergens should be prioritized when designing platforms for allergy diagnosis, especially for asthma, in tropical settings. Other studies have found that sensitization to Blo t 21 and Blo t 5 is frequent in asthmatic patients [8,13], but this is the first time they are analyzed together in a single sample population, under a case/control approach.

Several efforts have been done to get a representative panel for HDM allergy diagnosis in the tropics [15,16]. Our work contributes to this task with the identification of two allergens (Blo t 21 and Blo t 5) of possible importance in asthma. Of course, we cannot rule out that other allergens in the platform used in this study have clinical impact, but their effects may be mediated by non-IgE mediated immune pathways [17,35].
We found that the frequencies of patient’s sensitization to Blo t 5 and Blo t 21 were 24.4% and 24% respectively, which are lower than those found in Singapore (Blo t 5 = 45% and Blo t 21 = 57%) and Brazil with rates close to 80% for both allergens [13,16]. This could be due to differences in the technique for detecting specific IgE, but other reasons could be mentioned, such as population’s age, inclusion criteria, differences in exposure, isoform variations and even differences in the recombinant product used. In the same study of Gao et al, in a small sample of 42 B. tropicalis individuals with persistent allergic rhinitis, whose clinical characterization included nasal provocation test with B. tropicalis, IgE reactivity to Blo t 21, determined by serology and SPT, was 92-93% [12]. However, the frequency of IgE-reactivity to Blo t 21 in a less clinically characterized sample of 494 allergic individuals in Singapore was 57.9%. Also, it is important to remark that Colombia, due to the Andeans, has different climates and B. tropicalis is more abundant in the warmer areas. In fact, in cities of high altitude, such as Bogotá, contrary to Dermatophagoides spp., B. tropicalis has not been detected in house dust [36,37]. This could explain why the rate of sensitization to Blo t 21 and Blo t 5 in Barranquilla, a city at sea level was almost twice (40%) than the average rate of all cities. These differences have been also observed in the rates of IgE response to the complete B. tropicalis extract [22].

Other studies have evaluated the cross-reactivity between Blo t 5 and Blo t 21, reporting marked differences in their results [11,38]. In this work, we have confirmed that in our population, there is moderate cross-reactivity between Blo t 5 and Blo t 21 that may be high for some subjects, therefore it is possible that part of the IgE-binding frequency exhibited by both allergens is due to this phenomenon. Structural studies support that Blo t 5 and Blo t 21
may share epitopes [38]. However, our analyses show that they are independently associated with asthma. Besides, since there is evidence suggesting that there is no cross-reactivity between these two molecules and *Ascaris* spp. extract [13], their use, together with other *B. tropicalis*-specific allergen is highly recommended for diagnosing *B. tropicalis* allergy.

Since *Blo t 2* was the most common sensitizer in asthma patients, but also in controls, it was not associated with asthma presentation. Compared to *Der p 2* [26,39,40], there is less evidence about the intrinsic allergenic activity and biological functions of *Blo t 2*. However, other have reported that *Blo t 2* sensitizes a considerable proportion of HDM allergic patients. Reginald et al found that 36% of Singaporean allergic patients were positive to *Blo t 2* [14], a rate that coincides with data from Gabon and Colombia in children with recurrent wheezing or asthma [41,42]. However, its clinical importance in asthma or other allergic diseases have not been evaluated yet.

This is the first report of *Blo t 7* sensitizations in Latin America. Kidon *et al* found that *Blo t 7* is one of the most important sensitizers (57%) in Singaporean pediatric patients, after *Blo t 21* (56%) and *Blo t 5* (45%) and concluded that with the combination of these three recombinant components, the sensitivity to diagnose *B. tropicalis* sensitization is about 70% [16]. However, this was not replicated in our population where sensitization rates were low (<15%) and similar between patients and controls. Presence of isoforms and differences in geographical distribution may be considered, as it has been observed for other allergens [34].

Some limitations should be declared. The development of a multiplex assay to detect IgE may reduce optimal performance of the test for some antigens. Also, since this a
questionnaire-based study, there is a risk of bias selection for asthma and control groups. Frequency rates of sensitization may also be lower in this community-based study than in other reports where patients are recruited in well-characterized sample study from medical centers or specialized allergist services. This study was designed to evaluate general rates of sensitization to each allergen but is under-powered to report differences of sensitizations among cities. Although our results may be generalizable to other allergic diseases, it must be recognized that these associations were analyzed only in the context of asthma. Similar analyses may be performed in the context of allergic rhinitis.

In conclusion, Blo t 5 and Blo t 21 are associated with asthma in a case-control study. Our results support that both components should be included in molecular panels for allergy diagnosis in the tropics. Being a common sensitizer is not necessarily linked to clinical relevance, as it is probably the case of Blo t 2 in the context of asthma.

**Acknowledgments**

Thanks to all investigators of the Allergic Disease Prevalence study that gave rise to the initial research project where samples were collected.

This research was funded by Minciencias (Grant Number 803-2018), Minciencias/SGR (Grant Number BPIN2020000100405) and the University of Cartagena.
Social media

In this paper, we contribute to identify which Blomia tropicalis allergens are associated with Asthma in a case-control study. IgE responses to Blo t 5 and Blo t 21 are more frequent and intense in the disease group.
REFERENCES

15. Pinheiro CS, Silva ES, de Andrade Belitardo EMM, Pacheco LGC, Aguiar E, Alcantara-Neves NM, et al. En route to personalized medicine: uncovering distinct IgE reactivity pattern to house
33. Alvarez JRU. Obtenção, caracterização físico-química, imunológica e hipoalergização do alérgeno recombinante Blo t 2 visando o desenvolvimento de imunoterapia para alergia ao ácaro Blomia tropicalis.

J Investig Allergol Clin Immunol 2024; Vol. 34(3) © 2023 Esmon Publicidad
doi: 10.18176/jiaci.0892
### Tables

Table 1. Descriptive features of the study sample.

<table>
<thead>
<tr>
<th>Feature</th>
<th>Asthma cases (n = 272)</th>
<th>Controls (n = 298)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age in years [mean (SD)]</strong></td>
<td>28.0 (17.26)</td>
<td>28.8 (16.51)</td>
<td>0.68</td>
</tr>
<tr>
<td><strong>Males [n (%)]</strong></td>
<td>89 (32.7)</td>
<td>124 (41.6)</td>
<td>0.028</td>
</tr>
<tr>
<td><strong>City [n (%)]</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Barranquilla</td>
<td>30 (11.0)</td>
<td>30 (10.1)</td>
<td></td>
</tr>
<tr>
<td>Bogotá</td>
<td>149 (54.8)</td>
<td>168 (56.4)</td>
<td></td>
</tr>
<tr>
<td>Cali</td>
<td>47 (17.3)</td>
<td>51 (17.1)</td>
<td>0.98</td>
</tr>
<tr>
<td>Medellín</td>
<td>27 (9.9)</td>
<td>27 (9.1)</td>
<td></td>
</tr>
<tr>
<td>San Andrés</td>
<td>19 (7.0)</td>
<td>22 (7.4)</td>
<td></td>
</tr>
<tr>
<td><strong>D. pteronyssinus [n (%)]</strong></td>
<td>119 (43.8)</td>
<td>67 (22.5)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td><strong>B. tropicalis [n (%)]</strong></td>
<td>100 (36.8)</td>
<td>57 (19.1)</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>
Table 2. Sensitization to Blomia tropicalis components and asthma presentation: univariate logistic regression analysis.

<table>
<thead>
<tr>
<th>Allergen</th>
<th>Biochemical identity</th>
<th>Controls</th>
<th>Asthma</th>
<th>OR</th>
<th>95 CI</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Blo t 2</strong></td>
<td>ML-domain protein</td>
<td>69 (23.9)</td>
<td>74 (27.3)</td>
<td>1.20</td>
<td>(0.82 - 1.75)</td>
<td>0.35</td>
</tr>
<tr>
<td><strong>Blo t 5</strong></td>
<td>Unknown</td>
<td>48 (16.6)</td>
<td>66 (24.4)</td>
<td>1.62</td>
<td>(1.07 - 2.45)</td>
<td><strong>0.02</strong></td>
</tr>
<tr>
<td><strong>Blo t 7</strong></td>
<td>Lipid binding</td>
<td>38 (13.1)</td>
<td>39 (14.4)</td>
<td>1.11</td>
<td>(0.69 - 1.80)</td>
<td>0.67</td>
</tr>
<tr>
<td><strong>Blo t 8</strong></td>
<td>GST</td>
<td>36 (12.5)</td>
<td>34 (12.5)</td>
<td>1.01</td>
<td>(0.61 - 1.66)</td>
<td>0.98</td>
</tr>
<tr>
<td><strong>Blo t 10</strong></td>
<td>Tropomyosin</td>
<td>28 (9.7)</td>
<td>28 (10.3)</td>
<td>1.07</td>
<td>(0.62 - 1.87)</td>
<td>0.80</td>
</tr>
<tr>
<td><strong>Blo t 12</strong></td>
<td>Chitin binding</td>
<td>26 (9.0)</td>
<td>25 (9.2)</td>
<td>1.03</td>
<td>(0.58 - 1.83)</td>
<td>0.93</td>
</tr>
<tr>
<td><strong>Blo t 13</strong></td>
<td>Fatty acid binding</td>
<td>44 (15.2)</td>
<td>47 (17.3)</td>
<td>1.17</td>
<td>(0.75 - 1.83)</td>
<td>0.50</td>
</tr>
<tr>
<td><strong>Blo t 21</strong></td>
<td>Unknown</td>
<td>43 (14.9)</td>
<td>65 (24.0)</td>
<td><strong>1.81</strong></td>
<td>(1.18 - 2.77)</td>
<td><strong>0.007</strong></td>
</tr>
</tbody>
</table>
Table 3. Association of Blo t 21 and Blo t 5 sensitization with asthma presentation: multivariate logistic regression analysis.

<table>
<thead>
<tr>
<th>Predictor</th>
<th>aOR</th>
<th>95% C.I. aOR</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Blo t 21 model</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SES</td>
<td>1.01</td>
<td>0.98 - 1.04</td>
<td>0.59</td>
</tr>
<tr>
<td>Sex (male)</td>
<td>0.65</td>
<td>0.45 - 0.92</td>
<td>0.02</td>
</tr>
<tr>
<td>Age (in years)</td>
<td>1.00</td>
<td>0.99 - 1.01</td>
<td>0.41</td>
</tr>
<tr>
<td>City of residence</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(REF: San Andrés)</td>
<td>-</td>
<td>-</td>
<td>0.99</td>
</tr>
<tr>
<td>Barranquilla</td>
<td>1.13</td>
<td>0.50 - 2.53</td>
<td>0.77</td>
</tr>
<tr>
<td>Bogotá</td>
<td>1.06</td>
<td>0.55 - 2.06</td>
<td>0.86</td>
</tr>
<tr>
<td>Cali</td>
<td>1.10</td>
<td>0.52 - 2.31</td>
<td>0.81</td>
</tr>
<tr>
<td>Medellín</td>
<td>1.20</td>
<td>0.52 - 2.75</td>
<td>0.67</td>
</tr>
<tr>
<td><strong>Sensitization to Blo t 21 (%)</strong></td>
<td>1.83</td>
<td>1.18 - 2.83</td>
<td>0.007</td>
</tr>
<tr>
<td><strong>Blo t 5 model</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SES</td>
<td>1.08</td>
<td>0.78 - 1.50</td>
<td>0.63</td>
</tr>
<tr>
<td>Sex (male)</td>
<td>1.00</td>
<td>0.99 - 1.01</td>
<td>0.46</td>
</tr>
<tr>
<td>Age (in years)</td>
<td>0.63</td>
<td>0.44 - 0.90</td>
<td>0.01</td>
</tr>
<tr>
<td>City of residence</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(REF: San Andrés)</td>
<td>-</td>
<td>-</td>
<td>1.00</td>
</tr>
<tr>
<td>Barranquilla</td>
<td>1.06</td>
<td>0.47 - 2.41</td>
<td>0.89</td>
</tr>
<tr>
<td>Bogotá</td>
<td>0.99</td>
<td>0.50 - 1.95</td>
<td>0.97</td>
</tr>
<tr>
<td>Cali</td>
<td>1.05</td>
<td>0.49 - 2.25</td>
<td>0.90</td>
</tr>
<tr>
<td>Medellín</td>
<td>1.08</td>
<td>0.47 - 2.51</td>
<td>0.86</td>
</tr>
<tr>
<td><strong>Sensitization to Blo t 5 (%)</strong></td>
<td>1.61</td>
<td>1.05 - 2.47</td>
<td>0.03</td>
</tr>
</tbody>
</table>

*REF: reference; SES: socioeconomic status
Table 4. Distribution of specific IgE levels to Blomia tropicalis components in asthma and control groups.

<table>
<thead>
<tr>
<th>Allergen</th>
<th>Control group</th>
<th>Asthma cases</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Geometric mean</td>
<td>IQR</td>
<td>Geometric mean</td>
</tr>
<tr>
<td>Blo t 2</td>
<td>0.082</td>
<td>0.070 - 0.089</td>
<td>0.087</td>
</tr>
<tr>
<td>Blo t 5</td>
<td>0.078</td>
<td>0.067 - 0.082</td>
<td>0.088</td>
</tr>
<tr>
<td>Blo t 7</td>
<td>0.076</td>
<td>0.066 - 0.078</td>
<td>0.075</td>
</tr>
<tr>
<td>Blo t 8</td>
<td>0.074</td>
<td>0.064 - 0.075</td>
<td>0.074</td>
</tr>
<tr>
<td>Blo t 10</td>
<td>0.071</td>
<td>0.063 - 0.072</td>
<td>0.071</td>
</tr>
<tr>
<td>Blo t 12</td>
<td>0.071</td>
<td>0.063 - 0.073</td>
<td>0.071</td>
</tr>
<tr>
<td>Blo t 13</td>
<td>0.076</td>
<td>0.064 - 0.077</td>
<td>0.079</td>
</tr>
<tr>
<td>Blo t 21</td>
<td><strong>0.077</strong></td>
<td><strong>0.065 - 0.079</strong></td>
<td><strong>0.090</strong></td>
</tr>
</tbody>
</table>

IQR: interquartile range
Figures

Figure 1. Sensitization to *Blomia tropicalis* allergens in asthmatic and control subjects. Bar graphs with frequency rates of sensitization in both groups. Comparisons were done with Chi-square test. * $P < 0.05$
Figure 2. Allergen specific IgE values in asthma patients and control subjects.

Strip plots showing individual values of specific IgE to each molecular component. Log-transformed values were used to improve distribution and visualization. *P <0.05 (Mann-Whitney test was used).
Figure 3. Cross-reactivity assessment of Blo t 5 and Blo t 21.

A) Correlogram of specific IgE values to the eight allergens included in the *B. tropicalis* panel. Color bar indicates the r coefficient. B) Scatter plot depicting the correlation between log specific IgE to Blo t 21 and Blo t 5 in the analyzed sample (n=570), regression line and its 95% CI is shown. C) ELISA inhibition curves between Blo t 21 and Blo t 5 using serum pools of double-sensitized asthmatic patients. D) Point-inhibitions of IgE binding in 5 allergic patients. Each serum was inhibited with the homolog and heterologous condition as well as BSA as a non-related negative control.
Figure 4. UpSet plot representing co-sensitization patterns among the analyzed *Blomia tropicalis* allergens.

Intersection sets are presented separately for asthma (A) and control subjects (B). Values above vertical bars indicate the number of subjects sensitized to the allergens highlighted below with a black circle and connecting lines. First column indicates the number of subjects not responding to any allergen. Horizontal bars (left of each graph) indicate the number of subjects responding to each allergen (set size) and are organized in a descendent manner.