

# Differences in Molecular Sensitization Profiles Between Spanish and Latin American Mite-Allergic Patients

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## ■ Abstract

**Objective:** To analyze sensitization to *Dermatophagoides pteronyssinus* and to investigate the association between diagnostic findings and clinical severity in 218 allergic patients from 2 continents.

**Methods:** Mite-allergic patients were recruited by allergology departments in Latin America (n=88: Colombia, Costa Rica, and Guatemala) and Spain (n=130). All patients had allergic rhinitis with or without asthma and positive skin prick test results to *D. pteronyssinus*. Specific IgE levels to *D. pteronyssinus*, *Dermatophagoides farinae*, Der p 1, Der p 2, and Der p 23 were quantified using ImmunoCAP (Thermo Fisher Scientific). The allergenic profile was also determined by Western blotting. A comparative statistical analysis was performed using GraphPad software.

**Results:** Patients most frequently recognized Der p 2 (79%), followed by Der p 1 (73%) and Der p 23 (69%). The percentage of patients with asthma increased with the number of sensitizations; however, no statistically significant differences were found. Interestingly, patients with asthma presented the highest median levels of total IgE and specific IgE for *D. pteronyssinus* and molecular allergens, mainly Der p 2. Analysis of both populations revealed that Spanish patients were predominantly sensitized to Der p 2 (88.46%) and Der p 1 (83.84%), whereas Latin American patients were more sensitized to Der p 23.

**Conclusions:** Our data support the relevance of Der p 2 as the major allergen in mite allergy. A large percentage of patients are sensitized to this allergen, which plays a key role in the development of asthma. Sensitization to Der p 23 was more relevant in Latin America.

**Key words:** House dust mites. Sensitization profile. Component-resolved molecular diagnosis. Specific IgE. Allergic asthma. *Dermatophagoides pteronyssinus*.

## ■ Resumen

**Objetivo:** Asociar el patrón de sensibilización a *Dermatophagoides pteronyssinus* y la gravedad de la enfermedad alérgica en 218 pacientes procedentes de dos continentes distintos.

**Metodología:** Se reclutaron 88 pacientes alérgicos en países de Latinoamérica (Colombia, Costa Rica y Guatemala) y 130 de España (Europa). Todos los pacientes presentaban rinitis con o sin asma alérgica y resultados positivos a *Prick test* de *D. pteronyssinus*. Se analizaron los niveles de IgE específica en suero frente al extracto completo de *D. pteronyssinus*, *D. farinae* y a los alérgenos moleculares Der p 1, Der p 2, y Der p 23 mediante ImmunoCAP. El perfil alergénico completo se determinó mediante Westernblot. Los datos obtenidos se analizaron estadísticamente mediante el software GraphPad.

**Resultados:** Der p 2 fue el alérgeno más reconocido por los pacientes (79%) seguido de Der p 1 (73%), y Der p 23 (69%). El porcentaje de pacientes con asma fue más elevado cuanto mayor era el número de sensibilizaciones que presentaban. Además, los pacientes asmáticos presentaron niveles más elevados de IgE total, e IgE específica al extracto de *D. pteronyssinus* y alérgenos moleculares, principalmente Der p 2, que los que no sufrían asma.

Respecto a la comparación de las dos poblaciones, los pacientes de España estaban sensibilizados predominantemente a Der p 2 (88,46%) y a Der p 1 (83,84%), mientras que la población latinoamericana presentaba mayor sensibilización a Der p 23.

**Conclusiones:** Nuestro estudio indica la alta importancia de Der p 2 en los pacientes asmáticos a consecuencia de su sensibilización a ácaros, además de la relevancia de Der p 23 en la población latinoamericana.

**Palabras clave:** Ácaros del polvo. Perfil de sensibilización. Diagnóstico molecular por componentes. IgE específica. Asma alérgica. *Dermatophagoides pteronyssinus*.

## Summary box

- **What do we know about this topic?**

House dust mite is a major cause of perennial respiratory allergy. Knowledge of the molecular profile of IgE-mediated sensitization is critical to the design of allergy immunotherapy products and might help predict the response to these products.

- **How does this study impact our current understanding and/or clinical management of this topic?**

This study supports the relevance of molecular allergens in the diagnosis of mite allergy. Our results indicate that Der p 2 is directly involved in the presence of asthma and highlight the relevance of Der p 23 in Latin America.

## Introduction

House dust mite is a major perennial allergen source and a significant cause of allergic rhinitis and asthma. The incidence of sensitization to mite allergens varies from 65 to 130 per million people in the general population worldwide [1]. The prevalence of sensitization to mites rises to 90%, with geographical variations [2].

Latin America is a tropical region with a high prevalence of *Dermatophagoides pteronyssinus* [3], as previously described in Lima (Peru) [4], Juiz de Fora (Brazil) [5], Cartagena and Santa Marta (Colombia) [6,7], and Caracas (Venezuela) [8]. Other studies in Cartagena, Colombia, and Florida, USA showed that sensitization to the species *D pteronyssinus* and *Blomia tropicalis* was associated with acute asthma in both adults and children [9]. In the case of Costa Rica, *B tropicalis* is the most frequent mite, followed by *Chortoglyphus arcuatus* and *D pteronyssinus* [10]. In Spain, 43% of patients with allergic rhinoconjunctivitis are sensitized to mites. In the specific case of Catalonia, the percentage rises to 71% [11].

To date, 41 allergen groups from domestic mites have been identified [12], the most relevant in terms of prevalence being group 1 (Der p 1 and Der f 1), group 2 (Der p 2 and Der f 2), and Der p 23 in the case of *D pteronyssinus* [13]. Component-resolved molecular diagnosis in allergy enables recognition of individual sensitization patterns. However, the relationship between the prevalence of molecular sensitivity and clinical relevance remains to be clarified [12,13]. Therefore, the present study aimed to analyze the main differences in sensitization to house dust mite between Spain and Latin America based on molecular diagnosis and to correlate respiratory symptoms with the molecular sensitization profile, focusing on the immunodominant allergens Der p 1, Der p 2, and Der p 23.

## Materials and Methods

### Study Population

A total of 218 unrelated mite-allergic patients were selected, recruited, and diagnosed in Spain (n=130) at the Allergy Service of Hospital Clinic in Barcelona and in 3 countries in Latin America (n=88), namely, Colombia

(n=30), Guatemala (n=28), and Costa Rica (n=30). The annual average temperature and humidity in the different areas were, respectively, as follows: Barcelona 16°C (5°C-28°C) and 70% (53%-76%); Cali 23°C (19°C-29°C) and 76% (70%-90%); Guatemala City 18°C (13°C-25°C) and 77% (73%-84%); San Jose 19°C (17°C-27°C) and 75% (72%-92%).

Mite-allergic patients fulfilled the following criteria: presence of rhinitis/rhinoconjunctivitis and/or asthma, a positive skin prick test result for *D pteronyssinus*, and no previous treatment with allergen-specific immunotherapy.

Patients were classified according to the frequency and severity of clinical symptoms based on the GEMA 5.0 Asthma Guidelines [14] and modified ARIA criteria [15]. Informed consent was obtained from each patient, and ethical approval for the study was obtained from the Ethics and Research Committee of Hospital Clinic, Barcelona and the ethics committees of the other participating hospitals.

A serum sample from peripheral blood was isolated from each patient, identified with a code label, and stored at -20°C until use.

### Skin Testing

All patients underwent skin prick testing for *D pteronyssinus* (LETI Pharma S.L.U., Madrid, Spain) at each hospital. Saline (0.9%) and histamine (10 mg/mL) were included, respectively, as negative and positive controls. A wheal was considered positive if the diameter was greater than 3 mm.

### IgE Detection and Allergenic Profile

Total serum IgE levels and specific IgE levels for *D pteronyssinus* and *Dermatophagoides farinae* were determined using an IgE enzyme immunoassay (ImmunoCAP, Thermo Fisher Scientific). Furthermore, the sensitization profile to 3 individual molecular allergens, Der p 1, Der p 2, and Der p 23, was also analyzed following the manufacturer's guidelines. Total IgE titers were expressed in international units per unit volume (kU/L), while sIgE levels were quoted in kU<sub>A</sub>/L with a cut-off value  $\geq 0.35$  kU<sub>A</sub>/L.

Allergenic profile was investigated using immunoblot. One hundred micrograms of lyophilized native allergenic *D pteronyssinus* extract (LETI Pharma) was loaded in an SDS-PAGE gel with 2.67% C, 15% T acrylamide under reducing conditions. Proteins from the gels were transferred

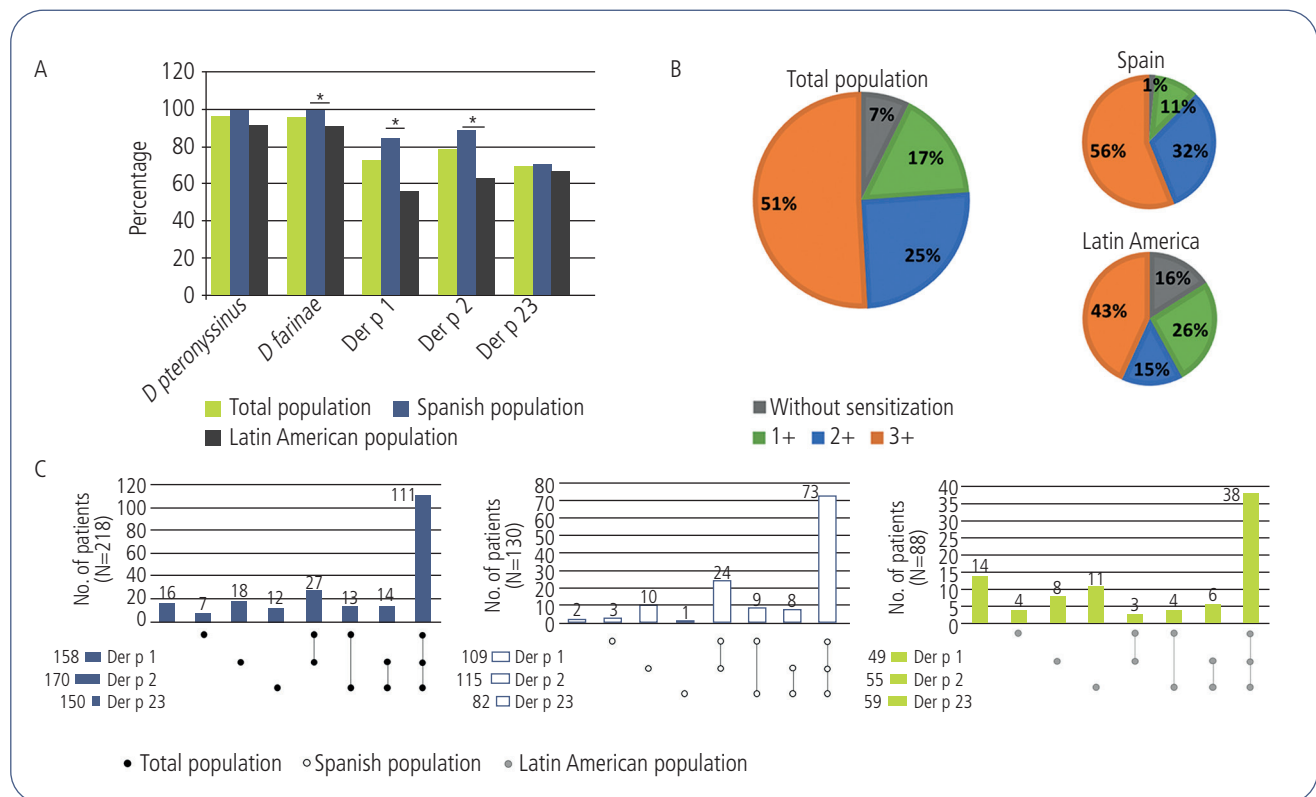
to an Immobilon1-P membrane (Millipore). The membrane was then incubated overnight with sera from allergic patients. Afterwards, the membrane was washed and incubated with monoclonal antihuman-IgE-PO (Ingenasa). Finally, the reaction was developed with Clarity Western ECL Substrate (Bio-Rad Laboratories).

**Statistical Analysis**

Data were analyzed using GraphPad Prism 7. Demographic features were quoted following standard descriptive means. Frequency was analyzed using the Fisher exact test, normality tests were performed for each variable, and comparisons of

**Table 1.** Demographic and Clinical Characteristics of the Study Population.

	Total population	Spain	Latin America
<b>Demographic characteristics</b>			
No.	218	130	88
Mean (SD) age, y	32.71 (10.91)	35.16 (11.04)	29.05 (9.66)
Female/male	58%/42%	58.46%/41.54%	56.8%/43.2%
<b>Clinical characteristics</b>			
Rhinoconjunctivitis	99%	99%	100%
Asthma			
	32%	41%	18%
	Intermittent/persistent mild 55%	Intermittent/persistent mild 43%	Intermittent/persistent mild 94%
	Persistent moderate/severe 45%	Persistent moderate/severe 57%	Persistent moderate/severe 6%



**Figure 1.** A, Percentage of individuals sensitized to *Dermatophagoides pteronyssinus*, *Dermatophagoides farinae*, and molecular allergens. \*Statistically significant differences by the Fisher exact test ( $P$  value  $< .05$ ). B, Percentage of individuals sensitized to 0, 1, 2, or 3 main allergens of *D pteronyssinus*. C, Number of patients sensitized to Der p 1, Der p 2, or/and Der p 23.

quantitative variables were compared using the Mann-Whitney test. A *P* value of <.05 was considered statistically significant.

## Results

### Study Population

The demographic and clinical parameters are summarized in Table 1. Most of the patients included in the study were females (58%). Mean age was 32.7 years (range, 18-66 years). Most individuals had rhinoconjunctivitis, whereas 32% had asthma.

### Differences in Sensitization to Molecular Allergen Between Populations

Ninety percent of the study population were sensitized to *D pteronyssinus* extract. Regarding molecular components, patients most frequently recognized Der p 2 (79%) followed closely by Der p 1 (73%) and Der p 23 (69%). Analysis of both populations revealed that patients from Spain were sensitized predominantly to Der p 2 (88.46%) and Der p 1 (83.84%), whereas Latin American patients were more sensitized to Der p 23 than to Der p 1 and Der p 2 (Figure 1A). Most patients were sensitized to the 3 major allergens (51%). Twenty-five percent were sensitized to 2 allergens (Figure 1B), mainly to Der p 1 and Der p 2, both in the total population and in Spain (Figure 1C). However, monosensitization to Der p 23 was more frequent in Latin American populations (12%) (Figure 1C).

Table 2. Immunoblot Analysis Against *Dermatophagoides pteronyssinus* Extract.<sup>a</sup>

Band, kDa	Prevalence of sensitization, %		
	Total study population	Spanish population	Latin American population
12	6.4	2.3 <sup>b</sup>	12.5
15	75.7	86.2 <sup>b</sup>	60.2
20	6.9	7.7	5.7
25	11.0	9.2	13.6
26	7.3	5.4	10.2
31	16.1	16.9	14.8
39	7.8	5.4	11.4
45	5.0	4.6	5.7
50	1.8	2.3	1.1
57	3.7	1.5	6.8
62	1.8	1.5	2.3
86	0.9	0.0	2.3
114	1.4	0.8	2.3
127	0.5	0.0	1.1
138	0.5	0.0	1.1

<sup>a</sup>Percentage of sensitization against specific bands in the immunoblot.

<sup>b</sup>Statistically significant differences between Spanish and Latin American populations (*P*<.05).

Eighty percent of the total population recognized at least 1 band of *D pteronyssinus* extract in the Western blotting analysis. A total of 12 different bands of sIgE recognition were detected at 12, 15, 20, 25, 26, 31, 39, 45, 50, 57, 62, and 114 kDa. Bands at 15 kDa and 31 kDa were the most frequently recognized by the whole population (75.7% and 16.1%, respectively) (Table 2) (Supplementary Figure 1). When populations were compared, statistically significant differences were obtained in recognition of the 12 and 15 kDa bands. The 12-kDa band was more frequently recognized by Latin American patients (12.5%). The 15 kDa band was more frequently recognized by Spanish patients (86.2%) than by Latin American patients (60.2%).

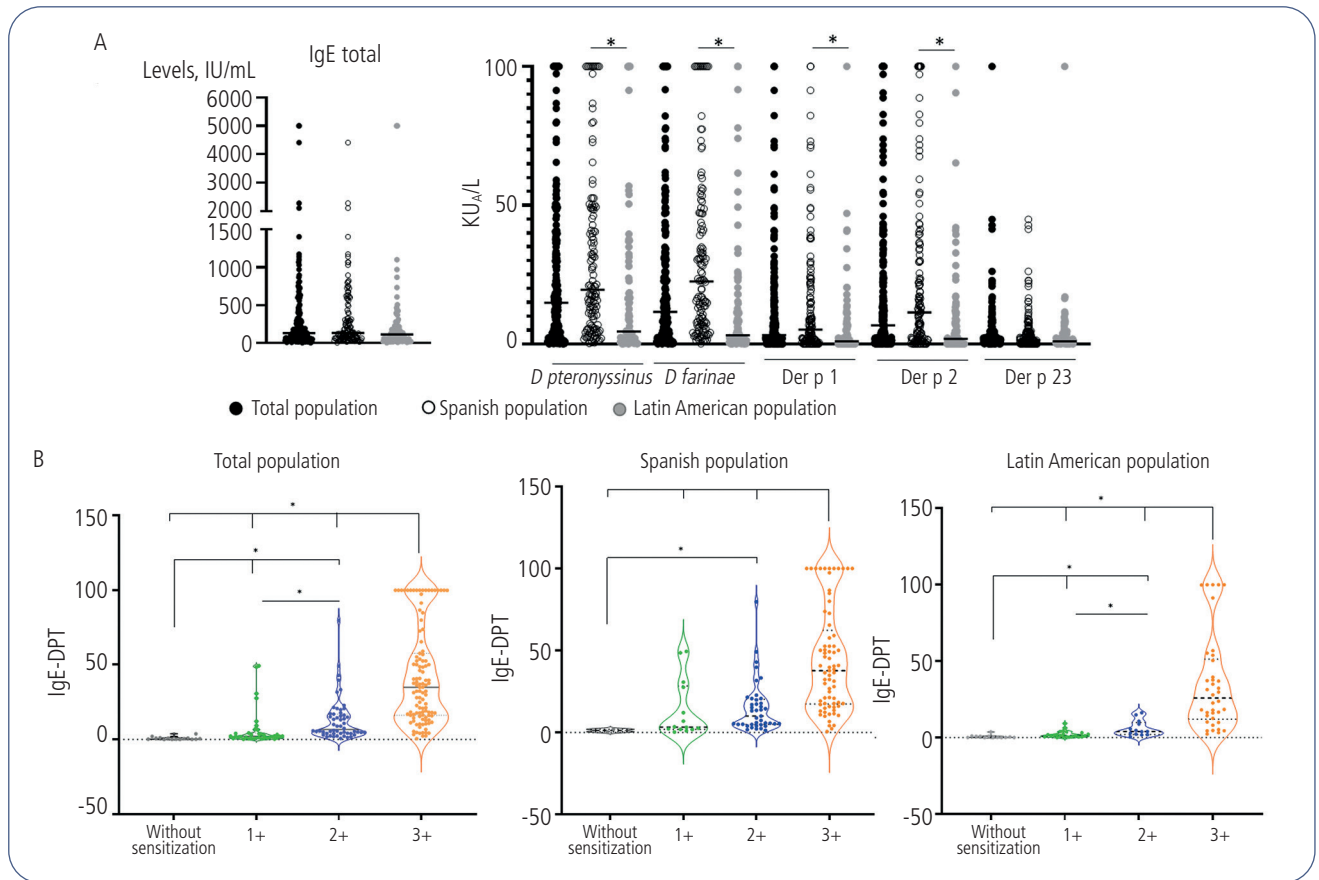
Median total IgE levels were 130 (57.85-302.5) kU<sub>A</sub>/L, with no significant differences between the populations. Median levels of *D pteronyssinus* and *D farinae* in the total population were 14.80 and 11.60 kU<sub>A</sub>/L, respectively. Regarding molecular allergens, levels of Der p 2 were the highest (6.74 [0.68-21.80]) and, as in the case of Der p 1 (3.20 [0.13-13.55]), were significantly higher in Spanish patients (Der p 1, 5.19 [1.11-21.45]; and Der p 2, 11.35 [1.95-32.55]) than in Latin American patients (Der p 1, 1.01 [0.02-6.78]; and Der p 2, 1.56 [0-9.10]). No statistically significant differences were observed between the populations for Der p 23 levels (Figure 2A). However, levels of IgE to *D pteronyssinus* were higher when patients were sensitized to a greater number of molecular allergens (Figure 2B).

Regarding the differences between the Latin American populations (Colombia, Costa Rica, and Guatemala), the immunoblot revealed several differences in terms of the number of bands of *D pteronyssinus* recognized by the serum samples (Supplementary Figure 1).

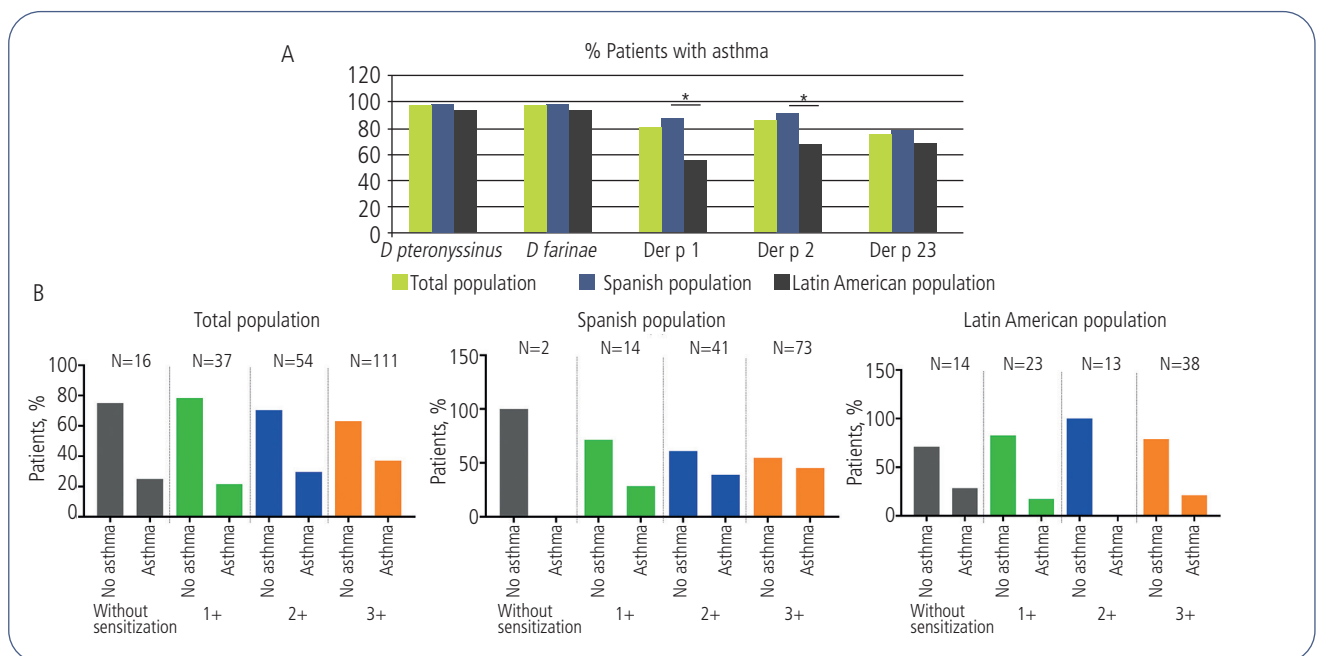
### Patients With Asthma Were More Frequently Sensitized and Had Higher Levels of sIgE

Regarding the relationship between the clinical symptoms and the sensitization profile, patients with asthma from Spain were more frequently sensitized to Der p 1 and Der p 2 than those from Latin America, with statistically significant differences (Figure 3A). People with asthma were more frequently sensitized in the total population, although the differences were not statistically significant (Figure 3B). Statistically significant differences were observed between patients with and without asthma and levels of total IgE. In addition, sIgE values were higher in patients with asthma than in those who did not have asthma, with the difference being statistically significant. Non-statistically significant differences were observed in the comparison of wheal sizes (Figure 4A). In Spanish patients, the only statistically significant differences observed were for levels of *D pteronyssinus* and *D farinae* allergen extract between patients with and without asthma, whereas levels of sIgE against molecular allergens were similar (Figure 4B) in both clinical groups. Regarding Latin American patients, no differences in sIgE levels were observed between the clinical groups (Figure 4C).

Analysis of correlations between age, sex, severity of allergic symptoms, and sensitization profiles revealed no positive correlations (data not shown).

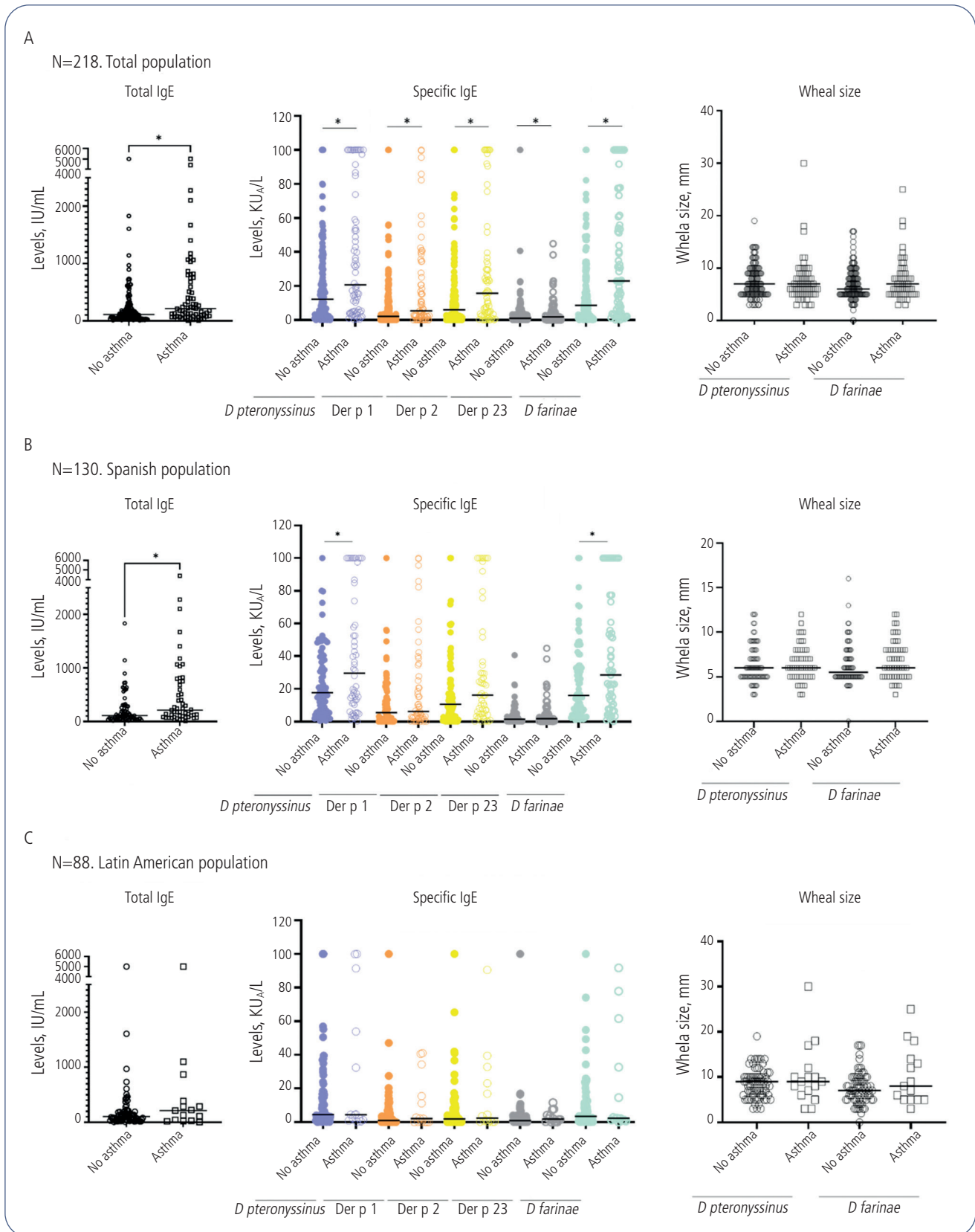


**Figure 2.** A, Total and specific IgE levels. Black lines indicate medians. \*Statistically significant differences by the Mann-Whitney test. B, Levels of sIgE to *Dermatophagoides pteronyssinus* in the different patient phenotypes. Negative indicates no sensitization; 1+, sensitization to 1 molecular allergen; 2+, sensitization to 2 molecular allergens; and 3+, sensitization to 3 molecular allergens. B, Specific IgE levels to *D. pteronyssinus* in the different patient phenotypes for the total population. Black lines indicate medians. \*Statistically significant differences by the Fisher exact test ( $P < .05$ ).



**Figure 3.** A, Percentage of patients with asthma according to allergen sensitization. B, Percentage of individuals with/without asthma in the different patient phenotypes. \*Statistically significant differences by the Fisher exact test, Negative indicates no sensitization; 1+, sensitization to 1 molecular allergen; 2+, sensitization to 2 molecular allergens; and 3+, sensitization to 3 molecular allergens.





**Figure 4.** A. Levels of total IgE/sIgE against allergens and wheal size depending on the clinical symptoms: patients with/without asthma. \*Statistically significant differences by the Mann-Whitney test ( $P < .05$ ). A, Total population. B, Spanish population. C, Latin American population. Black lines indicate the median.

## Discussion

The present study highlights the molecular sensitization pattern of *D pteronyssinus*, the most prevalent house dust mite, focusing not only on the prevalence of sensitization, but also on clinical impact and geographical factors.

Skin prick test positivity to *D pteronyssinus* was a main criterion, together with clinical symptoms, for including patients in the study population. Almost all the patients (99%) had allergic rhinoconjunctivitis and 30% had allergic asthma. Our analysis revealed that only 7% of patients with a positive skin prick result for *D pteronyssinus* extract did not have sIgE to the molecular allergens tested. However, most patients (51%) were sensitized to the 3 major allergens. This phenotype showed the highest levels of sIgE to *D pteronyssinus* extract and included the highest number of patients with asthma compared to patients sensitized to only 1 or 2 major allergens.

Analysis of the main differences between the 2 populations studied, Spain (n=130) and Latin America (n=88), revealed that the percentage of patients sensitized to only 1 allergen was higher than the percentage sensitized to 2 allergens in the Latin American population, while in the Spanish population, the percentage of patients sensitized to 2 molecular allergens was much higher. Der p 2 was the allergen most commonly recognized by the Spanish population, followed by Der p 1. In Latin America, Der p 23 was the most common allergen. Immunoblot analysis revealed Der p 2 and Der p 23 to be related to the 15-kDa band, the most frequently recognized band. These results are in line with those of previous studies carried out in Europe, which reported that sensitization to Der p 2 was more frequent than sensitization to Der p 1 [1,2,16-19], probably because Der p 2 is considered an adjuvant. It can cause a strong T<sub>H</sub>2 response in the bronchial epithelium and has a crucial role in activation of TLR4 owing to the similarity to myeloid differentiation antigen-2 (MD-2), a TLR-4 coreceptor [20,21]. Moreover, Der p 23 is considered a potent allergen [22,23] and a major allergen of house dust mite, as previously described in other populations from Spain, Austria, and Thailand [24-27]. It was considered a cause of persistent moderate-to-severe asthma in children [28]. In our study, levels of Der p 23 were significantly higher in patients with asthma than in those without, similar to Der p 1. The importance of this allergen is associated with cysteine protease activity. Its enzymatic activity could lead to the disruption of the epithelial barrier, thus promoting inflammation and induction of proinflammatory cytokines by the activation of protease-activated receptor-2 [20,29].

The differences in sensitization profiles between Colombia, Guatemala, and Costa Rica in the immunoblots could be explained by the differences in geographic, climatic, cultural, and genetic features between the 3 countries [3].

Analysis of more precise diagnostic techniques provides an important advance in the daily routine of allergy centers. Knowledge of allergen components and their prevalence could help health professionals to select the most appropriate immunotherapy for each patient [30,31]. However, the relationship between molecular sensitization pattern and clinical symptoms is complex and is influenced by both environmental exposure and genetic factors. In the present study, asthma was more frequent in Spanish patients than in

Latin American patients. In the total population, the percentage of patients with asthma increased with the number of sensitizations. However, no statistically significant differences were found. Patients with asthma presented the highest median levels of total IgE and sIgE to *D pteronyssinus* and molecular allergens. This finding was in line with those of previous studies [16,18,32-34]. The authors also mentioned that elevated sIgE responses to Der p 2 and Der p 23 were associated with the severity of the allergic disease. In our study, this finding indicated the presence of asthma, although no correlation with the severity of asthma was found.

The main limitation of the present study is the lack of information about the profile of sensitization to other allergens. More than 40 allergens have been described to date, although only 4 are commercially available (Der p 1, Der p 2, Der p 10, and Der p 23). Consequently, diagnosis of mite allergy is incomplete until the relevance of other allergens has been investigated. For that reason, there is an urgent need for new diagnostic tools that include other major allergens in order to improve diagnosis and support the composition of the extracts used for immunotherapy.

In conclusion, our data support the relevance of Der p 2 in mite allergy. This allergen is directly associated with the presence of asthma. The presence of sIgE to Der p 23 was more relevant in Latin America. Our data highlight the contribution of molecular diagnosis in the clinical study of mite-allergic patients.

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The authors declare that no funding was received for the present study.

## Conflicts of Interest

D Calzada and J Carnés are employees of LETI Pharma.

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