

Differences in molecular sensitization profiles between a Spanish and Latin American mite allergic patients

Running title: Molecular sensitization profile to mites

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Abstract

Objective: To analyze the sensitization pattern to *Dermatophagoides pteronyssinus* and to associate the diagnostic findings and clinical severity in 218 allergic patients from two different continents.

Methods: Mite allergic patients were recruited by the Allergology departments from 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*, *D. farinae*, Der p 1, Der p 2, and Der p 23 were quantified by ImmunoCAP system (ThermoFisher Scientific). Allergenic profile was also determined by western blot. Comparative Statistical analysis was performed by GraphPad software.

Results: Patients recognized most frequently Der p 2 (79%) followed by Der p 1 (73%), and Der p 23 (69%) allergens. The percentage of asthmatic patients increases with the number of sensitizations however none statistically significant differences were found. Interestingly, asthmatic patients presented the highest median levels of total IgE and specific IgE levels of *D. pteronyssinus* and molecular allergens, mainly Der p 2.

Analysing the two different populations, Spanish patients were predominantly sensitized to Der p 2 (88.46%) and Der p 1 (83.84%), whereas Latin American population were more sensitized to Der p 23.

Conclusions: Our data support the relevance of Der p 2 in mite allergy as the major allergen, with the high number of patients sensitized to it and its importance in the development of asthma. Sensitization to Der p 23 was more important in Latin America.

Key words: House dust mites, sensitization profile. Component-resolved molecular diagnosis of allergy. 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 Latino América (Colombia, Costa Rica y Guatemala) y 130 de España (Europa). Todos los pacientes presentaban rinitis con o sin asma alérgica y resultados positivos a test prick de *D. pteronyssinus*. Se analizaron los niveles de IgE específica en suero frente al extracto completo de *D. pteronyssinus*, y *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 western- blot. 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, y de 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 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, IgE específica, asma alérgica, *Dermatophagoides pteronyssinus*.

Summary box

– What do we know about this topic?

House dust mites are major sources responsible for perennial respiratory allergies. Knowledge of the molecular profile of IgE sensitization of patients is critical to the design of AIT products and might help predict the response to AIT.

– 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 the relevance of Der p 23 in Latin America population.

Introduction

House dust mites are 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 one of the tropical regions with higher prevalence of *D. pteronyssinus* [3] 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 *D. pteronyssinus* and *B. tropicalis* species were associated with acute asthma both in adults and children [9]. In the case of Costa Rica, *B. tropicalis* is the most frequent mite followed by *C. 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], being group 1 (Der p 1 and Der f 1), group 2 (Der p 2 and Der f 2) and Der p 23 in case of *D. pteronyssinus*, the most relevant in terms of prevalence [13]. Component-resolved molecular diagnosis in allergy allows 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 aim of the present study is to analyze the main differences of sensitization to house dust mites between two different populations from Spain and Latin America based on molecular allergen diagnosis and to correlate the 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 mites unrelated allergic patients were selected, recruited, and diagnosed in Spain (n=130) at the Allergy Service of Hospital Clinic in Barcelona and in three different countries of Latin America (n=88), including Colombia (n=30), Guatemala (n=28) and Costa Rica (n=30). Annual average temperature and humidity of different areas were respectively: Barcelona 16° (5-28) and 70% (53-76), Cali 23° (19-29) and 76% (70-90); Guatemala City 18° (13-25) and 77% (73-84); San Jose 19° (17-27) 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, according to 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 Ethical and Research Committee ethics committee of the Hospital Clinic of Barcelona and the other participating hospitals.

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

Skin testing

All subjects underwent skin prick testing for *Dermatophagoides pteronyssinus* (LETI Pharma S.L.u, Madrid, Spain) in each hospital. Saline (0.9%) and histamine (10 mg/mL) were respectively included as negative and positive controls. A positive wheal was considered 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 *D. farinae* were determined using an IgE enzyme immunoassay (ImmunoCAP system, ThermoFisher Scientific, Uppsala, Sweden). Furthermore, the sensitization profile to three individual molecular allergens -Der p 1, Der p 2 and Der p 23- was also analyzed following the endorsed manufacturer's guidelines. Total IgE titers were expressed in international units per unit volume (kU/L) while sIgE levels were quoted in kU_A/L with a cut-off value ≥ 0.35 kU_A/L.

Allergenic profile was investigated by immunoblot. A hundred micrograms of lyophilized *D. pteronyssinus* native allergenic extract (LETI Pharma) were loaded in Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis (SDS-PAGE) with 2.67% C, 15% T acrylamide under reducing conditions. Proteins of gels were transferred to an Immobilon1-P membrane (Millipore). Thereafter, the membrane was incubated overnight with different sera from allergic patients. Afterwards, the membrane was washed and incubated with monoclonal α -human-IgE-PO (Ingenasa, Madrid, Spain). Finally, the reaction was developed with Clarity™ Western ECL Substrate (Bio-Rad Laboratories).

Statistical analyses

Data was analyzed using GraphPad Prism 7 software. Demographic features were quoted following standard descriptive means. Frequency studies were analyzed by F Fisher, normalize studies were perform for each variable, comparisons of quantitative parameters, by Mann Whitney tests. A p-value of less than 0.05 was considered statistically significant.

Results

Study population

The demographic and clinical parameters are summarized in Table 1. Most of the subjects included in the study were females (58%). Mean age was 32.7 years, ranging from 18 to 66. The majority of individuals suffered rhinoconjunctivitis, whereas 32% were asthmatics.

Different molecular allergenic sensitizations among populations

Ninety percent of the study population was sensitized to *D.pteronysinus* extract. Regarding molecular components, patients recognized most frequently Der p 2 allergen (79%) followed closely by Der p 1 (73%), and Der p 23, 69%. Analyzing the two different populations, patients from Spain were predominantly sensitized to Der p 2 (88.46%) and Der p 1 (83.84%), whereas Latin American populations were more sensitized to Der p 23 than to Der p 1 and Der p 2 (Figure 1A). The majority of patients were polysensitized to the three major allergens (51%). The 25% were sensitized to two allergens (Figure 1B) mainly to Der p1 and Der p 2, both in the total population and in Spain (Figure 1C). However, monosensitization to Der p 23 was more important in Latin American populations (12%) (Figure 1C).

Eighty percent of the total population recognized at least a band of *D. pteronyssinus* extract in the western blot analysis. Globally, 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) (Figure 1Suppl). When populations were compared, statistically significant differences were obtained in recognition of the 12 and 15 kDa bands. The first one was more recognized by Latin American patients (12.5%). The 15 kDa was more frequently recognized by the Spanish population (86.2%) than in Latin American subjects (60.2%).

Median total IgE levels were 130 kUA/l (57.85 - 302.5) without significant differences among populations. Median levels of *D. pteronyssinus*, *D. farinae* in the total population were 14.80 and 11.60, 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 levels (3.20 (0.13-13.55)) were significant higher in the Spanish population (Der p 1: 5.19 (1.11-21.45) and Der p 2: 11.35 (1.95-32.55)) than in Latin American (Der p 1: 1.01 (0.02-6.78) and Der p 2: 1.56 (0-9.10)). None statistically significant differences were observed among population in Der p 23 levels (Figure 2A). On the other hand, IgE levels of *D. pteronyssinus* were higher when patients were sensitized to a higher number of molecular allergens (Figure 2B).

Regarding the differences among Latinamerican populations (Colombia, Costa Rica and Guatemala) the immunoblot showed several differences in terms of number of bands of *D. pteronyssinus* recognized by the serum samples (Figure 1 suppl).

Asthmatic patients presented higher number of sensitizations and higher levels of sIgE

Regarding the relationship between the clinical symptoms and the sensitization profile, asthmatic patients from Spain were more sensitized to Der p 1 and Der p 2 than asthmatic patients from Latin America, with statistically significant differences (Figure 3A). People with asthma presented higher number of sensitizations in the total population but non-statistical significances were observed among the group of study (Figure 3B). However, statistically significant differences were observed between non-asthmatic and asthmatic patients and levels of total IgE and every specific IgE measured were statistically higher in the asthmatic group compared with the non-asthmatics. Non-statistically significant differences were observed in the comparison of wheal sizes (Figure 4A). In Spanish population, only significant statistically differences were observed levels of *D. pteronyssinus* and *D. farinae* allergen extract between non-asthmatic and asthmatic patients, whereas levels of sIgE against molecular allergens were similar (Figure 4B) among both clinical

groups. Regarding Latin American patients no differences on sIgE levels among clinical groups was observed (Figure 4C).

Correlations among age, gender, severity of allergic symptoms and the sensitization profiles were performed, however no positive correlation was found (data no shown).

Discussion

The present study highlights the molecular sensitization pattern of *D. pteronyssinus*, the most prevalent house dust mite, focusing, not only in the prevalence of sensitization, but also in the 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. The 99% suffered allergic rhinoconjunctivitis and 30% allergic asthma. Our analysis revealed that only 7% of patients with a positive skin prick to *D. pteronyssinus* extract did not show sIgE to molecular allergens tested. However, the majority of patients (51%) was sensitized to the three major allergens. This phenotype of patients showed the highest levels of sIgE to *D. pteronyssinus* extract and presented the highest number of asthmatic patients compared to patients sensitized to only one or two major allergens.

Analyzing the main differences amongst the two populations studied, Spain (n=130) and Latin America (n=88), the percentage of patients sensitized to only one allergen was higher than the percentage sensitized to two allergens in the Latin American population, while in the Spanish population the percentage of patients sensitized to two molecular allergens was much higher. Derp 2 was the most recognized by the Spanish population followed by Der p 1. On the contrary, in Latin American population, Der p 23 was the most common one. In immunoblot analysis, Der p 2 and Der p 23 are related to 15 kDa band, the most recognized band. These results are in line with

previous studies carried out in Europe, reported that Der p 2 sensitization was higher than Der p 1 [1,2; 16-19]. This fact could be explained because Der p 2 is considered as auto-adjuvant. It can cause a strong Th2 response in the broncho epithelial route and has a crucial role of TLR4 activation due to the similarity to myeloid differentiation antigen-2 (MD-2), a TLR-4 co-receptor [20,21]. Moreover, Der p 23 is considered a potent allergen [22,23] and a major allergen of house dust mite, as was 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 population levels of Der p 23 were significantly higher in asthmatic patients than in non-asthmatic patients in a similar way of Der p 1 data. The importance of this allergen is related with the cysteine protease activity. Its enzymatic activity could lead the disruption of the epithelial barrier promoting inflammation and induction of proinflammatory cytokine by the activation of protease-activated-receptor-2 (PAR-2) [20,29].

Regarding the differences between the sensitization profiles between Colombians, Guatemalan and Costa Rican found in the immunoblots, could be explained by the differences in geographic, climate, cultural and genetic particularities among the three countries (3).

The analysis of more precise diagnostic techniques provides an important advance in daily routine in allergy centers. The knowledge about allergens components and their prevalence could help to select the most appropriate immunotherapy for each patient [30,31]. However, the relationship between molecular pattern sensitization and clinical symptoms is complex and influenced by both environmental exposure and genetic factors. In our case, asthmatic patients were more frequent in the Spanish population than in the case of Latin American patients. In the total population the percentage of asthmatic patients increases with the number of sensitizations, however no statistically significant differences were found. Asthmatic patients presented the highest median levels of total IgE and sIgE levels of *D. pteronyssinus* and molecular allergens. This finding was

in line with previous studies [16,18,32-34]. They also mentioned that elevated sIgE responses to Der p 2 and Der p 23 are related with severity of the allergic disease, in our study indicates the presence of asthma, however no correlation with the severity of this allergic disease 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 until now but just 4 (Der p 1, Der p 2, Der p 10 and Der p 23) are commercially available. This means that the diagnoses of mite allergic patients is incomplete until investigating the relevance of other allergens. For that reason, there is an urgent necessity of developing new diagnostic tools that include other major allergens that contribute to improving the diagnosis and support the composition of the extracts used for immunotherapy.

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

Conflicts of interest: D Calzada and J Carnés are employees of LETI Pharma.

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FIGURE LEGENDS

Figure 1. A) Percentage of individuals sensitized to *D. pteronyssinus*, *D. farinae* and molecular allergens. *Statistically significant differences by F Fisher analysis (p-value <0.05). 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 p2 or/and Der p 23.

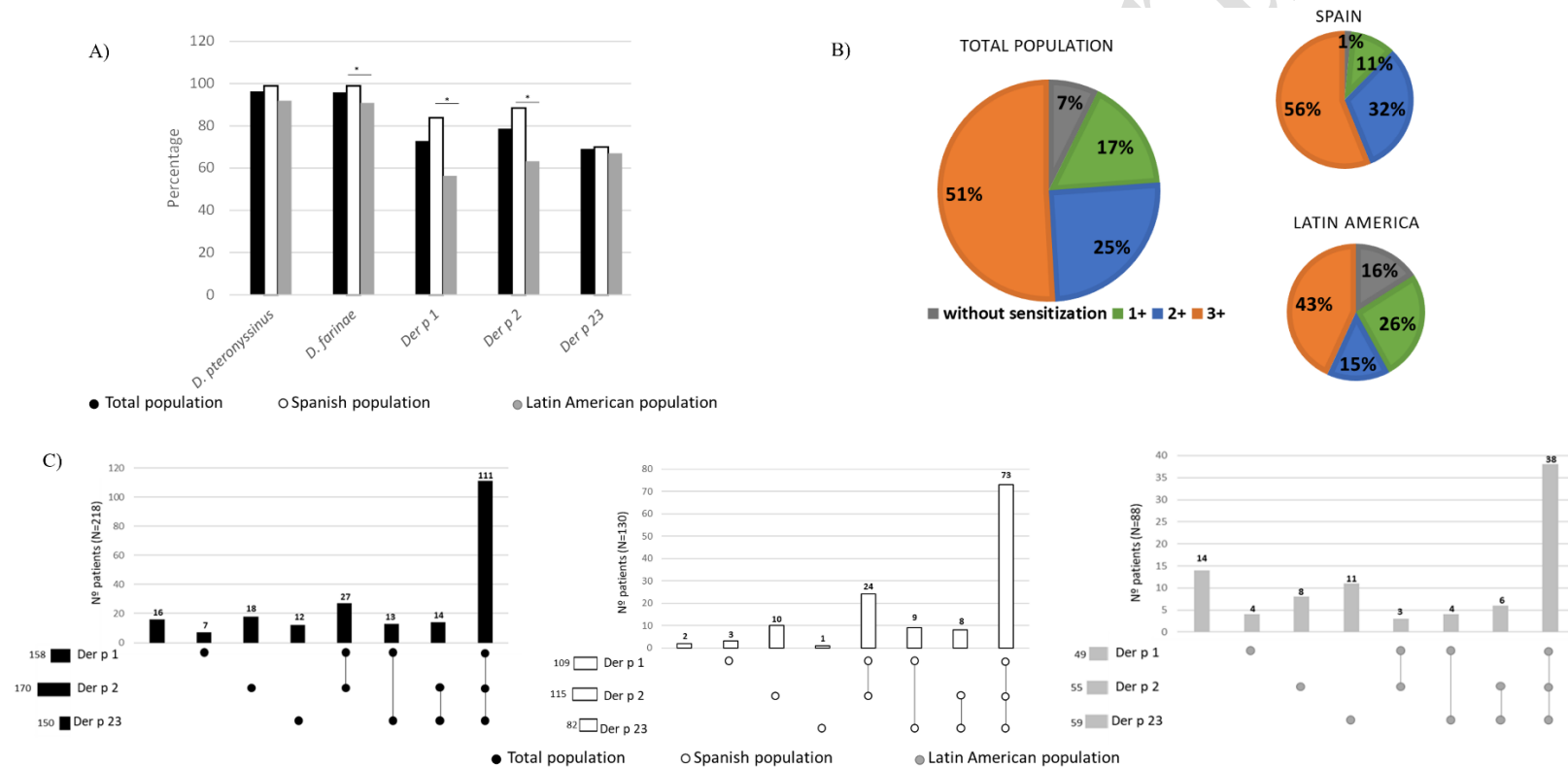


Figure 2. A) Total and specific IgE levels. Black lines mark medians. *Statistically significant differences by Mann Whitney tests. B) *Dermatophagoides pteronyssinus* sIgE levels in the different phenotype patients. Negative: without sensitization, 1+: sensitization to one molecular allergen, 2+: sensitization to 2 molecular allergens and 3+ sensitization to 3 molecular allergens B) Specific IgE levels to *D. pteronyssinus* in the different phenotype patients in the total population. Black lines mark medians. *Statistically significant differences by F Fisher analysis; (p-value <0.05).

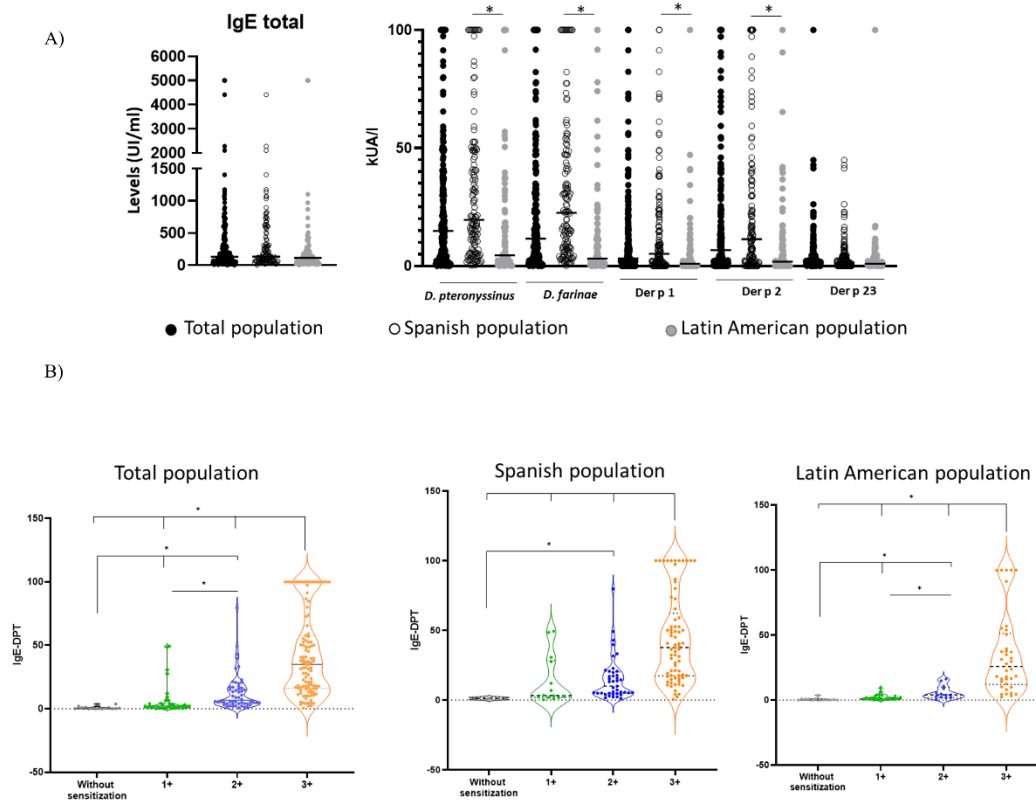


Figure 3. A) Percentage of asthmatic patients according to allergen sensitization. B) Percentage of individuals with/without asthma in the different phenotype patients. *Statistically significant differences by F Fisher analysis Negative: without sensitization, 1+: sensitization to one molecular allergen, 2+: sensitization to 2 molecular allergens and 3+ sensitization to 3 molecular allergens.

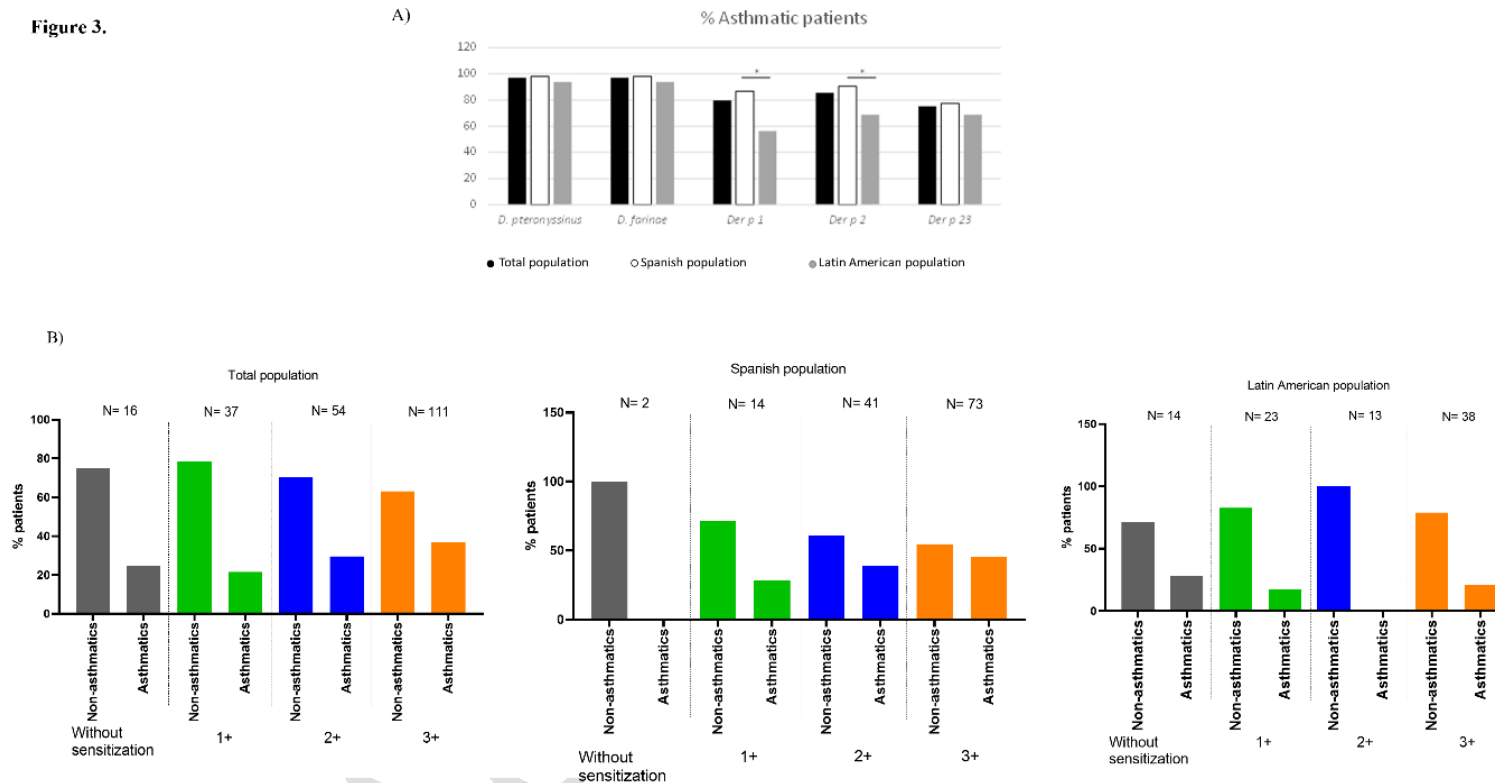


Figure 4. Levels of total IgE/ sIgE against allergens and size of papule depending on the clinical symptoms: non-asthmatic or asthmatic patients. *Statistically significant differences by Mann Whitney tests (p -value <0.05). A) Total population, B) Spanish population and C) Latin American population. Black lines mark media.

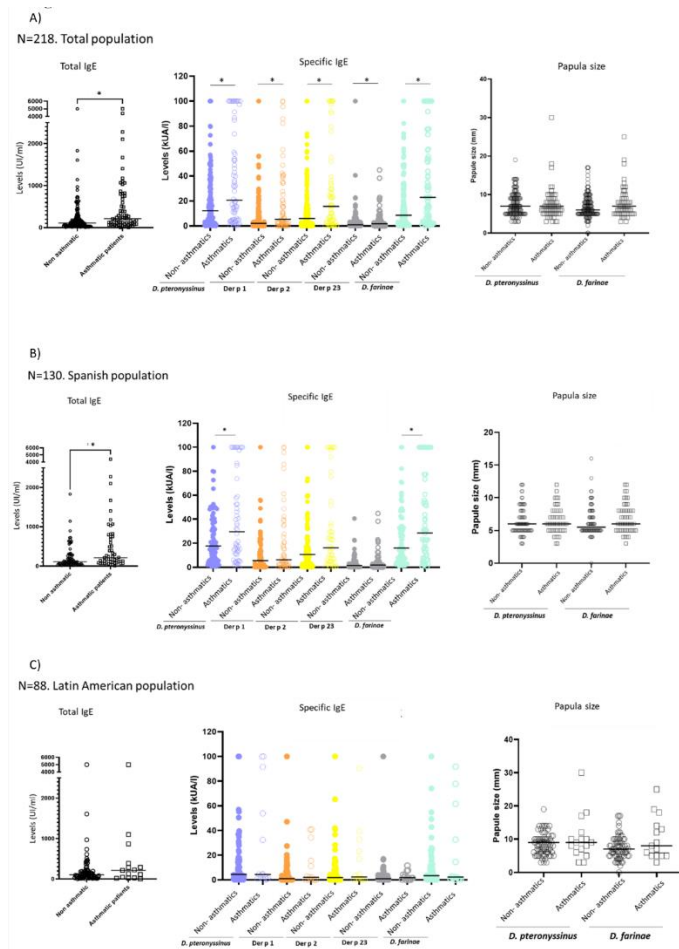


Table 1. Demographic and clinical characteristics of study population

		Total population				Spain				Latin America			
Demographic characteristics	N	218				130				88			
	Mean age (years)	32.71 ± 10.91				35.16 ± 11.04				29.05 ± 9.66			
	Gender female/male	58% /42%				58.46%/ 41.54%				56.8% / 43.2%			
Clinical characteristics	Rhinoconjunctivitis	99%				99%				100%			
	Asthma	32%	Intermitent/persistent –mild	55%	41%	Intermitent/persistent –mild	43%	18%	Intermitent/persistent –mild	94%			
			Persistent -moderate/-severe	45%		Persistent -moderate/-severe	57%		Persistent -moderate/-severe	6%			

Table 2. Immunoblot analysis against *D. pteronyssinus* extract. Percentage of sensitization against specific band in the immunoblot. * Statistically significant differences among Spanish and Latin American population (p-value <0.05).

Band (kDa)	Prevalence of Sensitization (%)		
	Total population of Study	Spanish Population	Latin America Population
12	6.4	2.3*	12.5
15	75.7	86.2*	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