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Mite Molecular Profile in the Th2-Polarized Moderate-to-Severe Persistent Asthma Endotype Subjected to High Allergen Exposure

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Airborne allergens · Allergen exposure · Asthma · *Dermatophagoides pteronyssinus* · Th2

Abstract
Background: The association among the IgE responses to prevailing groups of house dust mite (HDM) allergens in the concurrent asthma phenotypes has not been determined. ***Objective:*** The aim of the present study lays on a component-resolved diagnosis (CRD) model to investigate the mite molecular signature in subjects with type-2 inflammation asthma. ***Methods:*** We selected patients showing a clinically relevant sensitization to HDMs with moderate-to-severe persistent asthma. Skin prick test (SPT) with standardized mite extracts, a broad customized CRD serum sIgE panel including 9 *Dermatophagoides pteronyssinus* allergens and the related protein allergenic characterization, was investigated in all serum samples. ***Results:*** Ninety out of 93 (96.77%) patients with a positive SPT to HDM showed a concordant sIgE (≥0.35 kU_A/L) to the crude extract of *D. pteronyssinus*. Major allergens (Der p 2, Der p 23, and Der p 1) were present in >70% of all subjects, with mid-tier allergens (Der p 5, Der p 7, and Der p 21) reaching up to 51% in the present cohort. A complex pleomorphic repertoire of HDM molecules recognized by IgE was depicted, including 38 distinct profiles. ***Conclusions and Clinical Relevance:*** The proposed CRD panel approach, containing the most prevalent HDM allergens, appeared to be sufficient to obtain a precise *D. pteronyssinus* molecular diagnosis in asthmatics with a climate-dependent high-mite allergen exposure and coexisting sensitization. A dominant role of both major and mid-tier allergens has been confirmed in moderate and severe persistent asthmatics with the preponderant Th2-high endotype.

Introduction

Allergy is nowadays the most frequent immune disorder affecting almost 30% of the population worldwide, inducing inflammatory responses in the respiratory tract with a major impact on quality of life and associated health care cost [1, 2]. *Dermatophagoides pteronyssinus* was first identified in 1966 as the main allergen source

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responsible for respiratory allergies induced by the inhalation of house dust [3]. Nowadays, house dust mites (HDMs) are the most important source of perennial allergens influencing up to 85% of asthmatics from highly populated areas of the world and a risk factor for acute wheezing among asthmatic children [4, 5]. Approximately 10% of asthmatics present severe symptoms, defined by the requirement of high-dose inhaled corticosteroids and a second controller medication to prevent it from becoming "uncontrolled" or which remains "uncontrolled" despite this therapy [6]. These patients have the worse quality of life, both higher morbidity and mortality, and increased use of health care resources when compared with well-controlled asthmatics [7]. Moreover, HDM allergen immunotherapy (AIT) may currently benefit only patients with mild-moderate allergic asthma with the evidence of a clear relationship between symptoms and exposure to a specific allergen, considering severe asthma as a formal contraindication for the use of specific AIT, constituting a major independent risk factor for adverse reactions in this difficult-to-treat asthma group of highly sensitized subjects [8, 9].

The World Health Organization and the International Union of Immunological Societies (WHO/IUIS) Allergen Nomenclature Sub-committee currently includes up to 39 *Dermatophagoides* spp. allergens in the systematic nomenclature, with wide variations in prevalence rates for different countries [10, 11]. The *Dermatophagoides* spp. group 1, group 2, and group 23 allergens are considered immunodominant – binding 50–70% of the amount of IgE to HDM extracts – determined by gravimetric estimations or comparative titrations with proteins of known allergenicity. The medium or mid-tier allergens – group 4, 5, 7, and 21 allergens – bind IgE in 30–50% of mite-allergic patients and appear to account for most of the residual IgE binding of extracts [12].

The concept of component-resolved diagnosis (CRD) for molecular diagnosis was first introduced in 1999, performing a multi-allergen analyses enabling a comprehensive analysis of the patient's IgE-binding pattern to a large number of individual allergens [13, 14]. As the underrepresentation of certain allergens and/or competition by nonallergenic proteins is responsible for IgE levels measured with HDM allergen-extract-based ImmunoCAP™ being lower than those measured with molecular ImmunoCAP™, the replacement of allergen-extract-based tests for the detection of HDM sensitization with more specific molecular test has been suggested [15].

Quantification of atopic sensitization increases the specificity in relation to childhood asthma presence and severity [16] and persistence through adulthood bearing in mind that also the degree of asthma severity is associated with both sIgE levels and the number of sensitizations at the molecular level [17, 18]. Although different repertoires of specific HDM allergen sensitization have been described in persistent asthma, there is still insufficient evidence involving further HDM-sIgE responses in the etiopathogenesis of the type 2 asthma phenotype [19, 20]. The aim of the present study is based on a broader customized CRD model approach to investigate the mite molecular signature in a cohort of European moderate-to-severe asthmatics with a high perennial HDM exposure [21] dominated by subtropical weather conditions in Tenerife, Spain.

Methods

Subjects

We recruited 93 patients consecutively with a clinical diagnosis of moderate-to-severe persistent asthma with the Th2-high endotype (i.e., eosinophilic, with elevated total IgE and sIgE to airborne allergens and specifically mediated by the pro-inflammatory cytokines IL-4, IL-5, and IL-13) according to the recently updated 2020 Global Initiative for Asthma (GINA) Guidelines [22] from the Severe Asthma Unit and the outpatient allergy clinic at the Hospital Universitario de Canarias in (Tenerife, Spain), serving an area of 450,000 inhabitants under subtropical weather conditions, with mites as the most relevant local inhalant allergen [23]. All patients had to fulfill the following clinical criteria:

- Persistent asthma symptoms with seasonal exacerbations (spring, fall)
- Symptoms improve in altitude (>1,500 m), and
- Aggravation of symptoms by contact with household dust and indoor activities.

The following clinical data were collected from the patients' medical records: forced expiratory volume in the first second (FEV1), rhinitis and conjunctivitis diagnosis, treatments, a validated Asthma Control Test (ACT), and skin prick test (SPT) results. The subjects included in this study were divided into 2 groups based on their asthma severity (moderate and severe) according to the aforementioned GINA classification. Only subjects with an immediate positive SPT to HDM extract were included. Serum blood samples including blood eosinophil measurement were obtained from all participating subjects, identified with a code label, stored at −40°C, and thawed immediately before the in vitro analysis. Patients under treatment with AIT or monoclonal antibodies were excluded. Pregnant and breastfeeding women were excluded. The study was approved by the local ethics committee of our institution, and informed consent was signed by all subjects and parents/guardians for those participants aged <18 years.

Skin Prick Test

The SPT was performed according to the European Guidelines [24] with standardized extracts of *D. pteronyssinus* (Diater, Madrid, Spain). Histamine (10 mg/mL) and saline were used as positive and negative controls as usual. Following everyday practice,

Table 1. Descriptive statistics regarding basal comorbid conditions and associated asthma features of the studied population (n = 93)

	Moderate asthma	Severe asthma
	51 (54.83%)	42 (45.17%)
Age	27.8±1.9	34.62±2.3
<20 years	16 (31.37%)	11 (26.19%)
>20 years	35 (68.63%)	31 (73.8%)
Sex (F/M)	60.7%/39.3%	64.8%/35.2%
BMI	26.63	28.61
Allergic rhinitis	48 (94.11%)	39 (92.85%)
Atopic dermatitis	14 (27.45%)	11 (26.19%)
Food allergy	9 (17.64%)	8 (19.04%)
ACT	18	15
FVC	3,733 (90.3%)	3,221 (79.1%)
FEV1	3,030 (92.63%)	3,477 (76.24%)
SPT + *Dermatophagoides pteronyssinus*	51 (100%)	42 (100%)
Total IgE, IU/mL	611.5	522
sIgE *D. pteronyssinus*, kU_A/L	64.73	55.98
Eosinophils/mm^3 (serum)	370	420
Asthma onset at childhood	42 (82.35%)	34 (80.95%)
Family history of atopy	46 (90.19%)	36 (85.7%)

Median values are shown. SPT, skin prick test; ACT, Asthma Control Test; FVC, forced ventilatory capacity; FEV1, forced expiratory volume in the first second.

antihistamines were withdrawn a week prior to the SPT. The wheal diameters were measured after 20 min and those >3 mm were regarded as positive.

Mite Allergenic Extracts

Proteins from mite bodies of *D. pteronyssinus* were extracted in PBS, 0.01 M, pH 7.4, for 2 h at 5 ± 3°C. Both protein solutions were clarified by filtration and centrifugation (1 h at 16,000 *g*). Afterward, the isolated supernatants were ultrafiltrated against highly purified water (Ph. Eur. specification), sterile-filtered, frozen, and lyophilized.

SDS-PAGE/IgE Western Blot

Proteins from *D. pteronyssinus* extracts were analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), according to Laemmli [25], in 15% polyacrylamide gels under reducing conditions. Proteins were visualized by Coomassie Brilliant Blue R-250 staining and transferred to PVDF (Trans-Blot Turbo™; BioRad, Hercules, CA, USA). The binding of IgE antibody to allergens was analyzed by Western blot using individual patients' sera and anti-human IgE peroxidase conjugate (Southern Biotech, Birmingham, AL, USA). Chemiluminescence detection reagents (Western Lightning® Plus-ECL; Perkin Elmer, Waltham, MA, USA) were added following the manufacturer's instructions. IgE binding bands were identified using the BioRad Diversity database program.

Serological Analysis

Total IgE levels, sIgE to *D. pteronyssinus* (whole extract), and sIgE to Der p 1, Der p 2, Der p 5, Der p 7, Der p 10, Der p11, Der p 20, Der p 21, and Der p 23 were measured (ALEX MacroArray Diagnostics, Vienna, Austria) according to the manufacturer's instructions. In brief, ALEX is a multiplex array containing 282 reagents (157 extractive allergens and 125 molecular components). The different allergens and components were coupled onto polystyrene nano-beads, and then, the allergen beads were deposited on a nitrocellulose membrane, as published elsewhere [26]. Total IgE levels were expressed in international units per unit volume (IU/mL), and sIgE levels were expressed in kU_A/L. Values ≥0.35 kU_A/L were considered positive.

Statistical Analysis

Baseline and demographic characteristics will be summarized by standard descriptive summaries (medians and SDs for continuous variables and percentages for categorical variables). To compare differences, ANOVA, Kruskal-Wallis, and χ^2 tests are required for parametric continuous, nonparametric continuous, and categorical variables, respectively. A p value of <0.05 was considered statistically significant.

Results

Demographic Characteristics of Patients

Ninety-three Caucasian patients – 57 females and 36 males, median age 25.0 years – were recruited confirming their eligibility for the study (Table 1). All subjects who fulfilled the GINA criteria for moderate-to-severe Th2-

high persistent asthma had a positive skin SPT to *D. pteronyssinus*. Most of the subjects (81.65%) had their asthma onset during childhood or adolescence. Regarding atopic comorbidities, 87 patients (93.26%) suffered from allergic rhinitis, 25 subjects (26.88%) had atopic dermatitis, and 17 (18.27%) had food allergy (seafood, nuts, egg, and/or milk). The majority of patients (87.94%) had a known family history of atopy.

Total IgE and Blood Eosinophils

A quantitative analysis of serum total IgE was performed in order to evaluate the basal atopic status in the study population. The total IgE ranged from 13.28 to 14,770.0 IU/mL, with a median value of 662.5 IU/mL. The moderate asthmatic patients showed a median total IgE value (611.5 IU/mL) higher than the severe group (522.0 IU/mL). Blood eosinophils showed a median value of 390

Table 2. sIgE profiles of 9 *D. pteronyssinus* molecules examined in 93 subjects tested with microarray

n = 93	%	Number of molecules	Der p 1	Der p 2	Der p 23	Der p 5	Der p 7	Der p 21	Der p 10	Der p 11	Der p 20
7	7.50	0									
2	2.15	1			*						
1	1.07	1						*			
1	1.07	2			*			*			
5	5.37	3	*	*	*						
3	3.22	3		*	*	*					
1	1.07	3		*	*			*			
1	1.07	3	*		*				*		
1	1.07	3		*					*		*
1	1.07	3	*		*	*					
6	6.45	4	*	*	*			*			
3	3.22	4		*	*	*		*			
2	2.15	4	*	*	*	*					
1	1.07	4	*	*		*		*			
1	1.07	4			*	*		*	*		
1	1.07	4	*	*	*		*				
4	4.30	5	*	*	*	*		*			
2	2.15	5	*	*		*	*	*			
2	2.15	5	*	*	*	*	*				
2	2.15	5	*	*	*		*		*		
1	1.07	5	*	*	*		*	*			
1	1.07	5		*	*	*		*	*		
1	1.07	5		*	*	*	*	*			
1	1.07	5	*	*		*		*		*	
1	1.07	5	*	*		*				*	*
22	23.65	6	*	*	*	*	*	*			
2	2.15	6	*	*	*	*	*				*
2	2.15	6	*	*	*	*		*	*		
1	1.07	6	*	*	*	*	*		*		
1	1.07	6	*	*	*	*			*		*
1	1.07	6	*	*	*	*		*		*	
1	1.07	6	*	*	*	*	*				*
1	1.07	6		*	*	*		*	*	*	
4	4.30	7	*	*	*	*	*	*			*
1	1.07	7	*	*	*	*	*	*	*		
1	1.07	7	*	*	*	*		*	*		
3	3.22	8	*	*	*	*	*	*	*		*
1	1.07	8	*	*	*	*	*	*		*	*

Profiles are ordered by the increasing number of recognized *D. pteronyssinus* molecules. Major *D. pteronyssinus* allergens are shown in white, mid-tier allergens in grey and minor allergens in dark grey.

eosinophils/μL, with a higher median value (420.0 eosinophils/μL) in the severe asthma group than in those with the moderate asthma (370.0 eosinophils/μL) phenotype (Table 1).

Prevalence, sIgE Reactivity, and Individual Molecular Profile in Serum IgE in D. pteronyssinus Asthmatic Patients

Ninety patients (96.77%) were sIgE positive (≥0.35 kU_A/L) to the allergenic crude extract of *D. pteronyssinus* (0.5 to >100 kU_A/L, median 67.9 kU_A/L). Considering major allergens, 79 subjects (85.87%) were independently positive for either Der p 2 or Der p 23, while Der p 1 was present in nearly 74% of the studied samples. The median values of sIgE (kU_A/L) against Der p 2 were significantly higher ($p < 0.05$) than those against Der p 1 and Der p 23 (23.85, 10.91, and 13.8, respectively). In relation to the aggregation of allergens, the repertoire of molecules recognized by IgE was widely pleomorphic, including 38 distinct profiles in 93 subjects (Table 2; Fig. 1), with 6 specific molecules – *Der p 1*, *Der p 2*, *Der p 5*, *Der p 7*, *Der p 21*, and *Der p 23* – most frequently (23.65%) identified in both moderate and severe asthmatics. Der p 2, Der p 23, and Der p 1 exhibited as the most prevalent individual allergens, with 70.66% of patients sensitized to any of them, while mid-tier allergens – *Der p 5*, *Der p 7*, and *Der p 21* – showed a prevalence above 51%, and minor allergens such as Der p 10, Der p 11, and Der p 20 were present up to 14.95% in the serum samples of the present cohort. Eighty-seven of the subjects (94.57%) were positive to sIgE against Der p 1, Der p 2, Der p 5, Der p 7, Der p 21, and/or Der p 23. A higher prevalence of polysensitization to Der p 1, Der p 2, Der p 5, Der p 7, Der p 10, Der p 20, Der p 21, and Der p 23 ($p < 0.05$) was found in the moderate asthma patients compared to those in the severe group. Only 3 patients showed a monomolecular sIgE response to Der p 23 (2.15%) or Der p21 (1.07%), meanwhile no subjects were found to be solely sensitized to Der p 1, Der p 2, Der p 5, Der p 7, Der p 10, Der p 11, or Der p 20. Although 5 subjects (5.43%) from the severe asthma group (#148, #151, #153, #172, and #196) and 2 (#163 and #200) with moderate asthma symptoms (2.15%) had a positive sIgE response to the crude extract of *D. pteronys-*

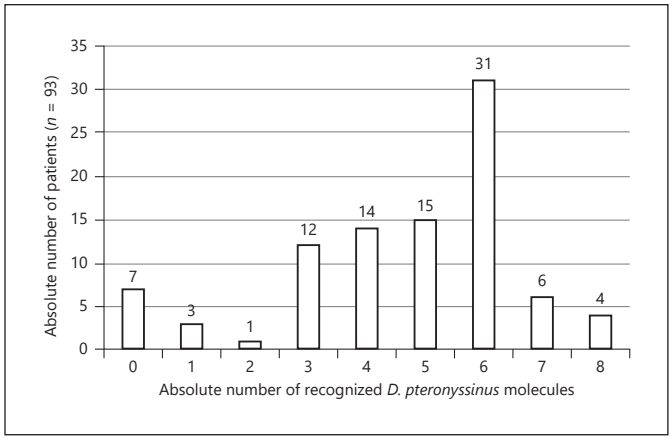


Fig. 1. Distribution of sIgE *Dermatophagoides pteronyssinus* molecules aggregated into different allergen profiles.

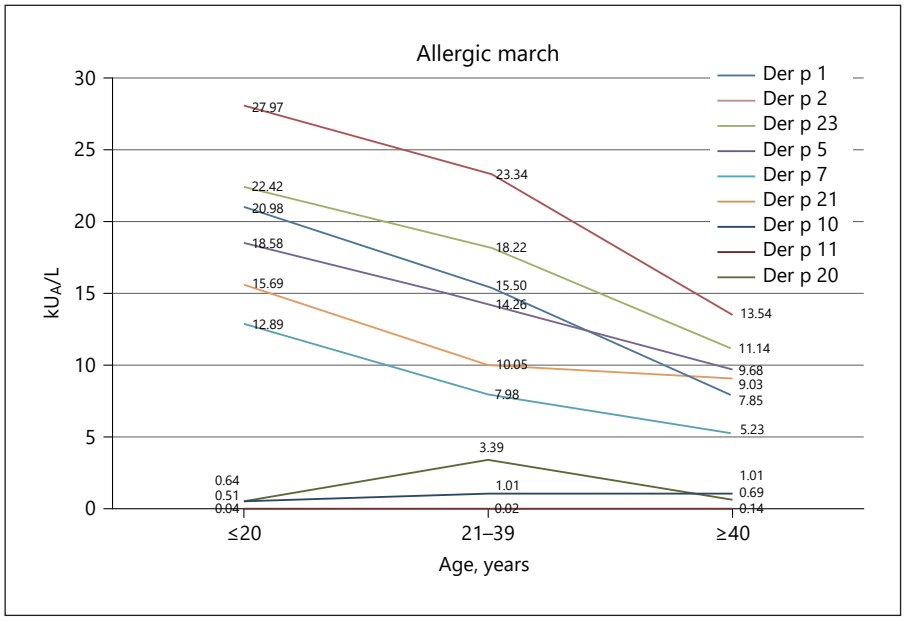
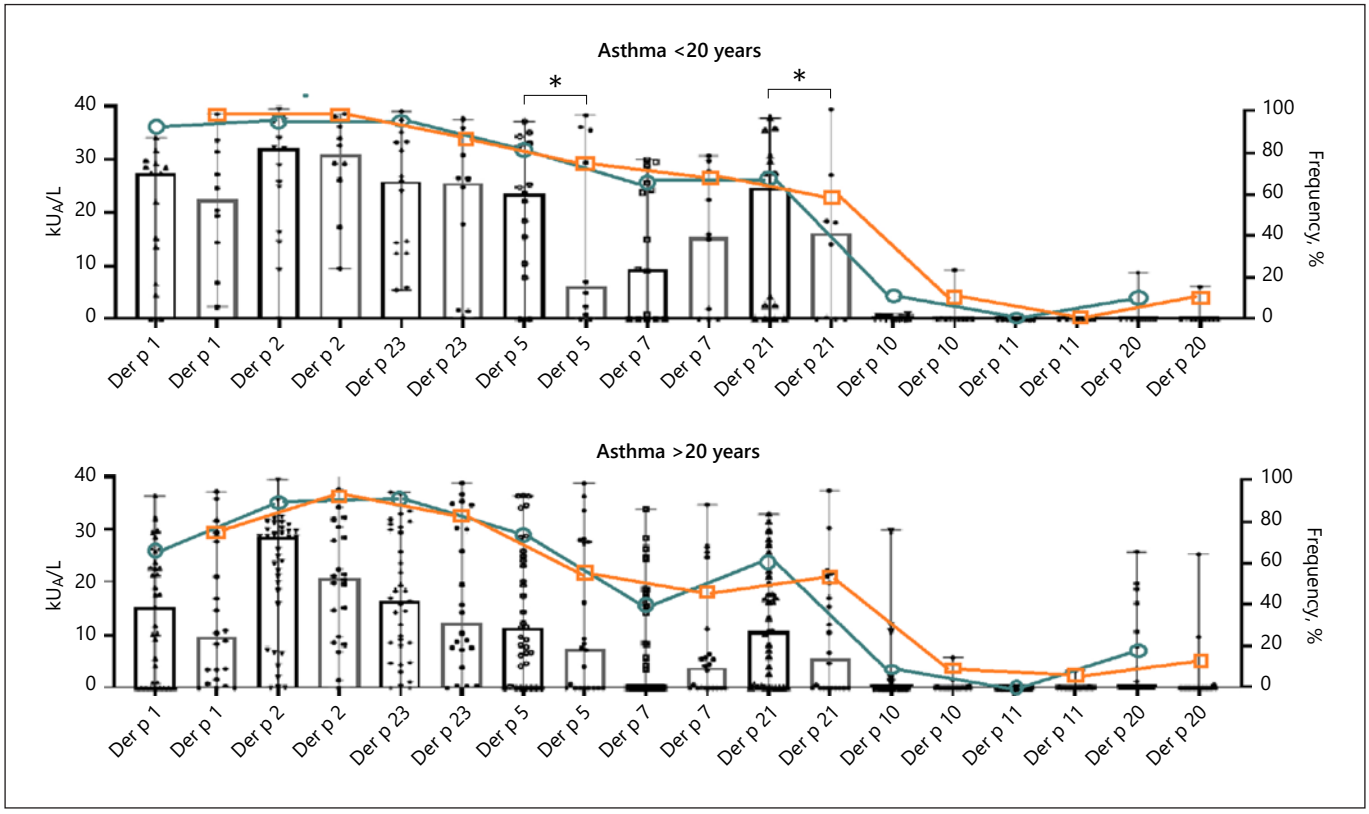


Fig. 2. Trending of predominance of the sIgE responses to 9 *Dermatophagoides pteronyssinus* molecules from <20 to >40 years of age.



Color version available online

Fig. 3. The quantitative average number of *Dermatophagoides pteronyssinus* molecules recognized by IgE antibodies was significantly increased in the younger asthmatic participants and the lowest in elder asthmatics aged >40 years. Median overall values of specific IgE of Der p 1, Der p 2, Der p5, Der p 7, Der p 10, Der p 20, and Der p 23 showed quantitative higher titers in the moderate asthma patients, only Der p 5 and Der p 21 were significantly higher in the moderate asthmatics (grey line bars) compared to those with the severe asthma symptoms (black line bars).

sinus; no detection to any of the 9 individual available allergens was found.

Relation of Sensitization Profile to sIgE with Age and Severity of Asthma

Specific IgE to crude extracts of *D. pteronyssinus* showed median values (kU_A/L) of 67.9 with a higher median level in the moderate asthma group than (49.01–33.16) those with the severe phenotype. The quantitative average number of *D. pteronyssinus* molecules recognized by IgE antibodies was increased in the younger participants (aged <20 years), intermediate in those aged from 20 to 40 years, and the lowest in elder asthmatics aged >40 years (Fig. 2). Although the ranking of predominance of the sIgE responses to most of the 9 molecules did not change in a relevant manner from <20 to >40 years of age, an increasing trend in terms of prevalence was exclusively observed for Der p 21 in those patients >40 years of age (Fig. 2).

However, with respect to the relation of the individual molecular sIgE to *D. pteronyssinus* and the severity of asthma, the median overall values of Der p 1, Der p 2, Der p5, Der p 7, Der p 10, Der p 20, and Der p 21 showed quantitative – but not statistically significant – higher titers in the moderate asthma patients (Fig. 3). The group of younger severe asthmatics (<20 years of age) showed significantly increased responses to Der p 2 ($p < 0.05$), and quantitative, although not statistically significant, higher total IgE and sIgE to Der p 1, Der p 7, and Der p 21 than their peers with milder-moderate asthma (Fig. 3).

SDS-PAGE/IgE Western Blot

Western blot of the selected patients with a sensitization to *D. pteronyssinus* showed different patterns of sen-

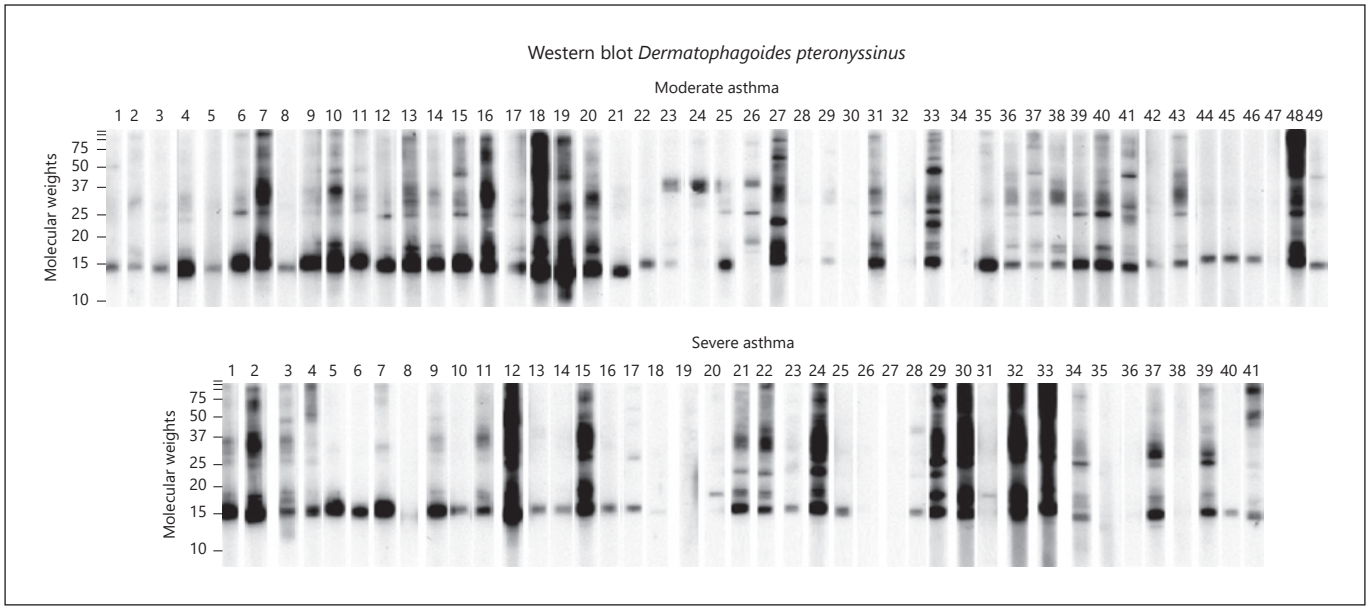


Fig. 4. Western blot of selected patients sensitized to *Dermatophagoides pteronyssinus.*

sitization (Fig. 4). A marked IgE binding intensity was found at 14–15 kDa for *D. pteronyssinus* in the majority of subjects (>80%), followed by significant recognitions around 24 kDa.

Discussion

Assessment of aeroallergen sensitization has been encouragingly recommended as a core biomarker for the classification of asthma, and in fact, a direct relationship between the degree of a given allergen sensitization and the likelihood of expression of asthma has been previously documented [27, 28]. The quantitative relationship between exposure to HDM allergens and symptoms in asthmatic patients is complex and influenced by both environmental and genetic factors, with many asthmatics simultaneously sensitized to >1 allergen, which makes determination of the contribution of a specific allergen to airway inflammation a difficult task [29]. Noteworthy, in the younger population, this association with allergen-specific IgE titters is specially marked, and increased sIgE in children reflecting a T2 profile [30]. Likewise, a key feature of mite sensitization in the tropics is the larger repertoire of specific mite allergens that the atopic individuals are sensitized to, in contrast to the predominant group 1 and/or 2 HDM-specific IgE responses in those more temperate regions with up to 80% of HDM allergic patients with specific IgE to these allergens [31, 32].

Previous investigations have shown that sIgE responses to an increased number of distinct mite allergens correlate with the complexity of the allergic phenotype and that both Der p 1 and Der p 2 are risk factors for multisystemic phenotypes of allergic diseases in Malaysia and China [33, 34]. Our study is, to our knowledge, the first to analyze the sIgE response to a comprehensive panel of 9 HDM allergens therein the spectrum of alike degree of severity – moderate to severe – in a cohort of asthmatics with the explicit Th2-high endotype, displaying an extremely heterogeneous repertoire of recognized molecules, and >92% of the asthmatic patients sensitized to at least 1 allergen. A complex polyclonal profile of *D. pteronyssinus*, comprising 6 molecules each, were identified in nearly 24% of the examined sera, exhibiting a markedly higher prevalence compared to former Asian and European cohorts – 7 and 0.8%, respectively – in those subjects sensitized to 6 different HDM molecules [31, 35, 36].

We confirmed a dominant role of sIgE sensitization to both Der p 2 and Der p 23 (85.87%) and Der p 1 (74%) among a selected population with moderate-to-severe persistent asthma in a subtropical part of Spain, showing a higher prevalence of IgE responses to Der p 1 and Der p 2 than those reported for Australia [37] (up to 77%) and Singapore [31] (63%), probably explained by a high pe-

rennial exposure to HDM and favored by the local weather conditions. In addition, the IgE response against Der p 2 and Der p 23 represented a larger fraction compared to Der p 1 and Der p 2 as shown by former reports in mainland Spain [38]. Mean specific IgE responses to Der p 2 and Der p 1 allergens were higher than those elicited by Der p 23 in terms of quantity, and moreover, the close correlation between these titers and the crude extract of *D. pteronyssinus* adds further supports to their dominant role in the HDM-sIgE response in this group of asthmatics.

In contrast to the findings of Becker and coworkers [39], with 5 out of 16 patients showing positive results for Der p 23 solely, Weghofer et al. [40] found that 6 of the 158 HDM allergic patients showed exclusive IgE reactivity to Der p 23 but not to any of the other 6 tested HDM allergens. Despite the high prevalence of sIgE response to Der p 23, only 2 subjects (2.17%) were exclusively sensitized to Der p 23 in our sample. The high prevalence (>85%) of Der p 23 sIgE confirmed the importance of this allergen component as a major HDM allergen supported by former research from Austria [41], Thailand [42], and Spain [43]. Batard et al. [44] equally reported that in temperate regions, 20–47% of 1,302 HDM allergic patients also showed sIgE to allergens from groups 4, 5, 7, 13, 15, 21, and 23. Our findings confirmed that mid-tier allergens – *Der p 5*, *Der p 7*, and *Der p 21* – reached a serodominant position with a prevalence >51%, while minor allergens – Der p 10, Der p 11, and Der p 20 – were present in nearly 15% of the studied samples.

Considering minor allergens, tropomyosin showed a detectable sIgE in 12 out 93 patients (12.90%) – particularly in those subjects aged <40 years – revealing a higher prevalence compared to former records from Asia [45, 46]. Our findings are not coincident with previous works [47], indicating that tropomyosin sensitization denotes true food allergy regardless of mite respiratory disease, as only 3 out of 12 sIgE Der p 10 responders (25%) had a confirmed seafood allergy. In line with earlier reports, the Der p 10-positive group exhibited significantly higher mean total IgE levels (1,373.02 IU/mL) than those patients without Der p 10 (662.5 IU/mL), suggesting a close connection between a broader sensitization profile and higher IgE levels [48, 49]. Additionally, although active scabies were discarded, Der p 20 sensitization was detected (8.60%), followed by Der p 11 (5.37%) in a similar way to earlier data comprising respiratory HDM allergy (6%) [50, 51].

Given that the proteolytic activity of Der p 1 seems more important at an early age and that Der p 2 takes the lead later in life, reflecting that exposure to Der p 1 in house dust is generally higher than exposure to Der p 2 [52, 53], it has been suggested that there might be similarities between the biological functions of the group 7 and group 2 mite allergens involving the binding of lipid substrates and that both allergens might co-opt innate immune responses into promoting allergenicity [54].

Interestingly, although younger individuals (aged <20 years) showed a higher mean level of sIgE to the crude extract of *D. pteronyssinus* and single allergens compared to elderly patients, we found that the sIgE response to Der p 2 was more prevalent and quantitatively higher than sIgE to Der p 1 and Der p 23 through all age groups. Furthermore, Der p 5, Der p 7, and Der p 21 were also present in both young and elderly individuals with the moderate-to-severe asthma. In such a way, despite not serodominant, their putative interactions with TLRs imply an influence over the progression of allergy, even though Der p 5 and Der p 21 embrace a major conformational IgE epitope-containing area located on similar portions of their structure, lacking relevant IgE cross-reactivity [55, 56].

In large birth cohort studies, asthmatic children were characterized by more complex molecular patterns of IgE sensitization to grass and mite molecules increasing with disease severity and age [35, 49]. Our data indicate that although the ranking of prevalence of the sIgE responses to most of the 9 molecules did not change in a relevant manner between <20 and >40 years of age, the quantitative average number of allergens recognized by sIgE was higher in the younger participants. This observation has been also previously addressed, as total IgE and sIgE were significantly decreased as a function of age in patients with allergic rhinitis or asthma, but not in those with atopic dermatitis, displaying a unique trend for each tested allergen [57, 58].

Asthma is usually more severe in patients who are exposed to higher allergen levels, and also the level of bronchial hyperresponsiveness correlates with both sIgE levels and the number of present sensitizations [59]. Although former asthma reports – despite including only a limited number of HDM allergens – have generalized more elevated IgE responses to Der p 2 [60] and Der p 23 [61, 62] in the severe degree of the disease, our findings showed that only Der p 2 was significantly higher in the severe younger asthmatics, with higher – but not statistically significant – total IgE and sIgE to Der p 1, Der p 7, and Der p 21 titers, compared to moderate asthmatics. Elucidating how the majority (>75%) of both moderate and severe asthma patients recognize 4 or more HDM molecules can

be intriguing; however, prior multivariate analyses have correlated the conjunction of different predisposing and/or protecting factors related to the environment – thus, natural exposure to mixtures of non-purified allergens and other elements influencing the effects of individual molecules – and the genetic background, to increasing pleomorphic mite-specific IgE responses [63, 64].

The present investigation has some limitations, as 7 subjects (7.5%) with a positive sIgE response to the crude extract could not be identified through our proposed molecular panel, besides other indoor allergens (i.e., storage mites, cats, and/or dogs) might have partially contributed to the severity of the respiratory symptoms. In this regard, Western IgE blots displayed a wide range of IgE-binding proteins, suggesting that more allergen components, other than the panel used in this study, may be involved. Furthermore, despite consistent with preceding investigations [34], only a limited number of patients were studied, making these findings challenging to match with other HDM-sensitive asthma populations, exposed to a different geography of mite allergens. Also, the role of co-sensitization to additional nearby prevalent mites such as *Blomia tropicalis* should be evaluated in future research concerning this population.

Our results showed that although >70% of the patients were sensitized to any of the major allergens, a combination panel including mid-tier allergens like Der p 5, Der p 7, and Der p 21 enhanced identification up to >94% of the moderate-to-severe mite-sensitized asthmatics. The role of both major and mid-tier *D. pteronyssinus* allergens needs further investigation not only in terms of prevalence but also in the pathophysiology underlying the type 2 inflammation in moderate-to-severe asthma to promote a genuine diagnosis, leading to a tailored mite AIT in the near future [65]. The proposed CRD panel approach appears to be sufficient to obtain a precise *D. pteronyssinus* molecular diagnosis in moderate-to-severe asthmatics with a marked mite allergen exposure, thus providing an enhanced depiction of the Th2-high asthma endotype.

Statement of Ethics

The study was approved by the local Ethics Committee of CEIC Hospital Universitario de Canarias, Tenerife, Spain, on October 30, 2017, with the reference number P.I.-2017/72. The institutional consent form was obtained by all subjects and parents or legal guardians for those aged <18 years taking part in the study.

Conflict of Interest Statement

All of the authors declare no competing interest concerning the manuscript.

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Author Contributions

R.G.-P., P.P.-G., and F.P. designed the study and wrote the manuscript. I.S.-M., P.P.-G., R.G.-P., V.M., E.M.-L., and C.A.-C. contributed to data collection. F.P., P.F., M.C., and M.A. carried out the in vitro investigations. F.P. and M.C. designed all enclosed figures in the original manuscript. P.P.-G., V.M., E.M.-L., and C.A.-C. performed the literature search. All authors contributed equally to the critical interpretation of the results. All authors approved the final version of the manuscript.

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