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ORIGINAL ARTICLE

Epidemiology and Genetics

Comparison of house dust mite sensitization profiles in allergic adults from Canada, Europe, South Africa and USA

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Abstract

Background: Sensitization to house dust mite (HDM) is a leading cause of allergic rhinitis and asthma. Despite more than 30 HDM-derived allergens having been identified to date, specific therapeutic approaches do not yet take into account the local sensitization profiles of patients. This study aimed to identify patterns of HDM sensitization in HDM-allergic adults living in distinct geographic areas, to inform the development of targeted diagnostic and therapeutic tools.

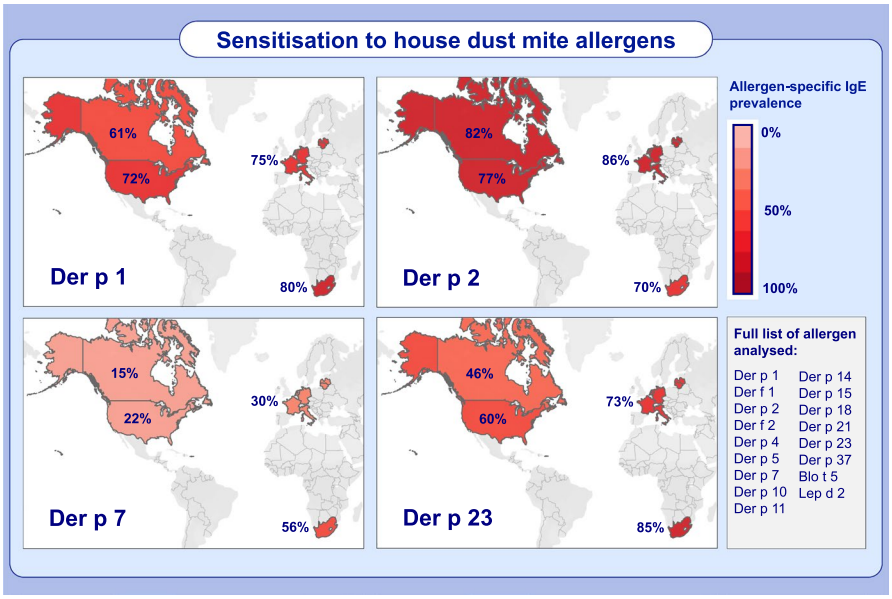
Methods: Serum samples from 685 HDM-allergic subjects from Canada, Europe, South Africa, and the USA were tested for levels of IgE specific for 17 micro-arrayed HDM allergens by ImmunoCAP Immuno Solid-phase Allergen Chip (ISAC) technology.

Results: The results confirmed significant geographical variability in sensitization patterns and levels of IgE. In all areas, the major sensitizers were the group 1 and group 2 allergens and Der p 23. Der p 23 was a frequent sensitizer: 64% of the subjects had IgE specific for Der p 23, and 2.3% were monosensitized to it. In South Africa, Der p 23 was the dominant HDM allergen (86% prevalence) and Der p 7 achieved major allergen status (56%). IgE sensitization to HDM was influenced by asthmatic status, levels of allergen exposure, age, race-ethnicity and smoking status, but not by BMI.

Abbreviations: AIT, allergen immunotherapy; Der p/Der f, *Dermatophagoides pteronyssinus/farinae*; GINA, global initiative for asthma; HDM, house dust mite; IgE, immunoglobulin E; ISAC, immuno solid-phase allergen chip; ISU-E, ISAC standardized IgE units; SPT, skin prick test.

Conclusion: Sensitization profiles to HDM allergens differ considerably among distinct geographic areas, with Der p 7 and Der p 23 being major sensitizers in South Africa. Such heterogeneity should be taken into account in the diagnosis and treatment of HDM-allergic patients.

KEYWORDS
allergen exposure, allergen microarray, Der p 23, Der p 7, IgE sensitization



GRAPHICAL ABSTRACT
Immunoglobulin E sensitization to major house dust mite allergens varies by geographic region. Der p 23 is the dominant house dust mite allergen in South Africa. Der p 7 is a major allergen in South Africa.

1 | INTRODUCTION

Except for very cold or arid regions, house dust mites (HDM) are found worldwide in human living environments.[1] They are a major cause of perennial allergic rhinitis, atopic dermatitis, and asthma, and are responsible for allergic symptoms in at least 1–2% of the global population.[2] HDM species *Dermatophagoides pteronyssinus* (Der p) and *Dermatophagoides farinae* (Der f) are the principal sources of sensitizing allergens.

To date, 39 HDM allergen groups have been identified and categorized by molecular profile and activity.[2–4] The group 1 and 2 allergens (ie, Der p 1 and Der f 1, and Der p 2 and Der f 2) are considered the most clinically relevant HDM allergens. Combined, they bind over 50% of HDM-related Immunoglobulin E (IgE) and they induce specific IgE in approximately 80% of mite-sensitive patients.[5,6] However, due to the considerable sequence homology within groups of HDM allergens of different species (eg, there is 80–90% homology within group 1 and within group 2 allergens from *D. pteronyssinus* and *D. farinae*), HDM-specific IgEs are usually not species-specific and they cannot distinguish between allergens of the same group derived from different species.[7]

Recently, Der p 23, a putative chitin-binding protein, has been added to the HDM immunodominant allergen list.[6,8,9] Accumulating evidence shows that Der p 23 elicits frequencies of sensitization similar to the other major allergens, although some studies have suggested that the levels of Der p 23-specific IgEs are lower compared to Der p 1 and 2.[6–13] Furthermore, not all studies agree on the prevalence of IgE to this allergen, potentially due to differences in the characteristics of the populations analyzed.[13] Allergens from groups 4, 5, 7, and 21 are mid-tier sources of sensitization. The remaining HDM allergen groups generate low or unknown prevalence of sensitization.[14]

The management of HDM-induced allergy and asthma has generally been palliative and limited to allergen avoidance and symptomatic relief medications.[15] Of late, guidelines have been amended to include HDM allergen immunotherapy (AIT).[16,17] AIT consists of repeated administrations of allergen extract, and it provides long-lasting relief from symptoms.[18–20] An important caveat is that the HDM extracts used in diagnosis and AIT are usually only standardized for group 1 and 2 allergens, leaving other important allergens under-represented or totally absent.[21–23] Since some patients are not sensitized to group 1 and 2 allergens,

this variability can introduce limitations and failures in the diagnosis and treatment of HDM-allergic subjects, as has been recently demonstrated in a clinical study setting and under real life conditions.[24,25]

As a result of component-resolved diagnosis, it is now possible to evaluate individual IgE reactivity profiles of HDM-allergic patients. In this study, we performed a comprehensive analysis of HDM allergen molecules in adult allergic cohorts from Canada, Europe, South Africa and the United States of America (USA). The evaluation of the importance of individual allergens in different geographic areas and of the factors affecting sensitization is key to develop targeted and effective diagnostic and therapeutic tools for HDM allergies.

2 | MATERIALS AND METHODS

2.1 | Subjects

Serum samples were obtained, at baseline, from 685 HDM-allergic subjects recruited in a multi-site Phase II clinical trial investigating a peptide-based treatment for HDM (HDM-SPIRE, ClinicalTrials.gov Identifier: NCT02150343). Allergic subjects were defined by a reliable history (at least 1 year) of moderate to severe rhinoconjunctivitis (sneezing, rhinorrhoea, itchy nose, nasal blockage and/or itchy eyes, red eyes, watering eyes and/or itchy ear/palate) on exposure to HDM which had required symptomatic treatment on at least one occasion during the last year, a positive skin prick test

TABLE 1 Demographic and geographic distribution of the study population

	USA[a] 356 (52%)	CANADA[b] 80 (13%)	EUROPE[c] 208 (30%)	SOUTH AFRICA[d] 41 (6%)	Total *N* (685)
Sex [*n* (%)]					
Male	113 (68.26)	37 (46.25)	93 (55.29)	14 (34.15)	275 (37.52)
Female	243 (31.74)	43 (53.75)	115 (44.71)	27 (65.85)	428 (62.48)
Race/Ethnicity [*n* (%)]					
Asian	21 (5.90)	2 (2.50)	4 (1.92)	4 (9.76)	31 (4.53)
Black/African American	64 (17.98)	2 (2.50)	1 (0.48)	3 (7.32)	70 (10.22)
Hispanic	31 (8.71)	5 (6.25)	8 (3.85)	0	44 (6.42)
White	209 (58.71)	67 (83.75)	14 (6.73)	8 (19.51)	463 (67.59)
Other/Mixed	10 (2.81)	2 (2.50)	2 (0.96)	25 (61.00)	39 (5.63)
Not collected	21 (5.90)	2 (2.50)	179 (86.06)	1 (2.44)	38 (5.55)
Age (years)					
Mean ± SD	38.5 ± 11.9	34.6 ± 12.0	33.5 ± 11.4	37.6 ± 14.8	36.5 ± 12.2
Median	37	31	31	35	35
Range	18–68	18–61	18–68	18–63	18–68
BMI (kg/m^2)					
Mean ± SD	29.1 ± 7.1	26.7 ± 5.4	24.6 ± 4.5	28.6 ± 6.1	27.4 ± 6.4
Median	27.4	26.1	24.0	30.2	26.2
Range	15.9–51.5	16.4–46.9	16.5–46.4	17.2–40.5	15.9–51.5
Sensitization status [*n* (%)]					
Monosensitized	102 (28.65)	22 (27.50)	51 (24.52)	18 (43.90)	193 (28.17)
Polysensitized[e]	254 (71.35)	58 (72.50)	157 (75.48)	23 (56.10)	492 (71.83)
Current asthma status [*n* (%)]					
Non-asthmatic	232 (69.25)	58 (74.36)	127 (34.87)	34 (85.00)	451 (69.60)
Asthmatic	103 (30.75)	20 (25.64)	68 (65.12)	6 (15.00)	197 (30.40)
Smoking status [*n* (%)]					
Current/Former smoker	69 (20.60)	19 (24.36)	38 (19.49)	15 (37.50)	141 (21.76)
Never smoked	266 (79.40)	59 (75.64)	157 (80.51)	25 (62.50)	507 (78.24)

[a]From 25 states: Alabama (7), California (100), Connecticut (3), Florida (6), Georgia (11), Illinois (11), Kentucky (3), Massachusetts (1), Maryland (27), Maine (1), Minnesota (8), Missouri (11), North Carolina (14), New Jersey (17), New York (5), Ohio (9), Oklahoma (6), Oregon (21), Pennsylvania (15), Rhode Island (4), Texas (31), Virginia (9), Vermont (8), Washington (20), Wisconsin (8).

[b]From 2 provinces: Ontario (30) and Quebec (50).

[c]From 6 countries: France (1), Germany (78), Italy (9), Latvia (22), Lithuania (43), Netherlands (7).

[d]From 2 provinces: Kwazulu-Natal (5) and Western Cape (36).

[e]The most frequent co-sensitizations were grass (43%) and cat (30%).

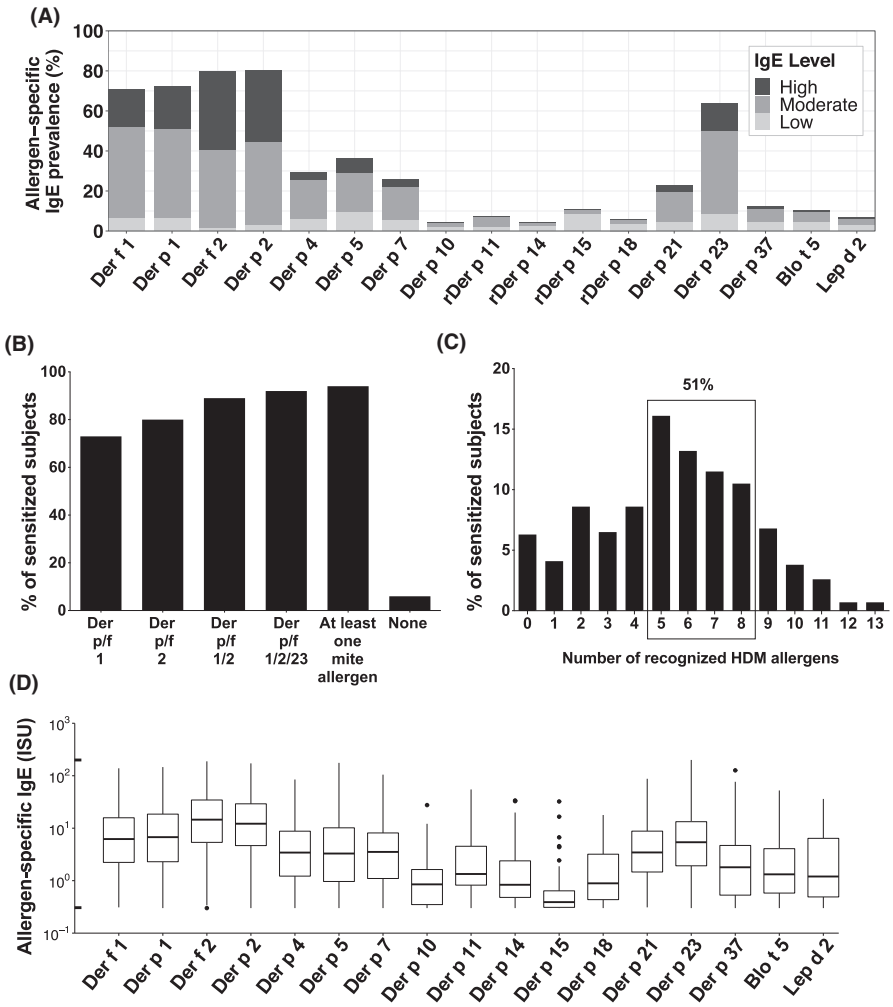


FIGURE 1 Frequencies of IgE responses to individual HDM allergens. (A and B) Prevalence of IgE sensitization (y-axis: percentages) to individual (A) and combinations (B) of HDM allergens (x-axis). In (A), the stacked columns show the percentage of individuals with high (ISU≥15, in black), moderate (ISU<15, ≥1, in dark gray) or low (ISU<1, ≥0.3, in light gray) IgE levels. (C) Frequency of allergic subjects with polysensitization to HDM allergens. The gray box indicates the number of allergens recognized by more of 50% of the study population. (D) Levels of IgE specific for 17 HDM allergens (y-axis, $\log^{10}$ scale). Boxes indicate the interquartile ranges, horizontal lines indicate the median values, and whiskers indicate maximum and minimum values, excluding outliers (showed as dots). Ticks on the right of the Y-axis indicate the range of the test (0.3–150 ISU). Statistically significant differences between Der p 23 and the other allergens' IgE levels are shown (*p ≤.05, ***p ≤ .001)

(SPT) to Der p or Der f allergens (wheal diameter ≥5 mm compared to the negative control), and Der p or Der f specific IgE ≥0.35 kU/L. Subjects were not eligible for the study if they had asthma requiring Global Initiative for Asthma (GINA) Step 3 (www.ginasthma.org) or higher treatment. Subjects were aged 18–70 years, and they were enrolled at 189 different sites in Canada, Europe, South Africa, and USA (Table 1). Race/ethnicity was self-reported and based on the US Census Bureau's questionnaire. Written informed consent was obtained from all individuals before sample collection. The study was approved by the ethics committees of all individual sites and was conducted in accordance with ICH GCP guidelines and the principles of the declaration of Helsinki.

2.2 | ImmunoCAP ISAC microarray

Serum samples were analyzed for the presence of specific IgE antibodies by Thermo Fisher Scientific (Uppsala, Sweden), using a customized version of the MeDALL allergen chip containing a panel of 17 HDM allergens (all recombinant except for natural Der p 1 and Der f 1), purified and characterized as previously described.[26] Results are reported in ISAC Standardized IgE Units (ISU-E, 0.3–150 ISU-E range). Subjects were considered sensitized to an allergen if IgE levels were >0.3 ISU-E.

2.3 | Dust sampling and allergen analysis

Subjects provided a dust sample from their home using a dust collection device attached to a vacuum cleaner (DUSTREAM™, Indoor Biotechnologies, Charlottesville, VA, USA), within 3 weeks from the blood sample collection. Analysis of the dust samples was conducted by Indoor Biotechnologies (Cardiff, UK and Charlottesville, VA, USA). Allergens were extracted from dust samples as previously described[27] and analyzed for the levels of the major allergens Der p 1 and Der f 1 using a multiplex immunoassay (MARIA™, Indoor Biotechnologies, Charlottesville, VA, USA).[28]

2.4 | Statistical analysis

RStudio (version 1.2.5033) and Prism (Graph Pad Software, version 7.0) were used for data visualization and statistical analysis. Differences in IgE allergen levels were assessed using the two-sample *t*-test (parametric data) and Wilcoxon test, or Kruskal-Wallis test (non-parametric data) followed by a pairwise Wilcoxon test for post-hoc analysis. Levene test was performed to determine normality, where $p \geq .05$ indicated normal distribution. Statistical significance was considered for p values ≤ .05. Differences in frequencies of IgE responses were

assessed using the multiple chi-square testing or the Fisher exact test with Bonferroni's correction, based on the number of observations.

3 | RESULTS

3.1 | Der p 23 is important for monosensitization in HDM-allergic subjects

Serum samples from 685 HDM-allergic subjects were tested for IgE specific for 17 HDM allergens. The majority of the subjects were female (62.5%), white (67.6%), with a mean age of 36 years (Table 1). In line with previous studies,[13,29] but with the important exception of the South African cohort (discussed below), the results of our analysis confirmed Der p 1, f 1, p 2, f 2 and p 23 as major allergens (prevalence above 50%), Der p 4, p 5, p 7, and p 21 as mid-tier allergens (prevalence between 20 and 50%), and the remaining allergens (Der p allergens from groups 10, 11, 14, 15, 18, and 37, Blo t 5 and Lep d 2) as minor allergens (prevalence below 20%) in the whole study population (Figure 1A). The proportion of subjects with IgE to group 1 allergens was lower than to group 2 allergens (73% and 80%, respectively, Figure 1B). The percentage of subjects with IgE to any of the major allergens was 92%, but only 89% of the subjects had IgE to at least one of the group 1 or group 2 allergens. Noticeably, 6% of the population did not show IgE for any of the 17 tested allergens, despite a positive SPT (Figure 1B,C).

Among the 73% of subjects with IgE to group 1 allergens, 97% were positive for both Der f 1 and p1 (12 subjects had exclusively anti-Der p 1 IgE, and 5 subjects only anti-Der f 1 IgE). Similarly, 99% of group 2 positive subjects had IgE to both Der f 2 and p 2 (1 subject had only IgE to Der f 2, and 5 subjects had only IgE to Der p 2). A strong cross-reactivity within group 1 and group 2 allergens was also suggested by the correlation between Der p 1 and f 1 ($r = .96$, $p < .001$), and between Der p 2 and f 2 ($r = .99$, $p < .001$) IgE levels (Data not shown).

The majority of the subjects recognized between 5 and 8 of the tested allergens (average=5.6, Figure 1C). The most frequent IgE profile included all 5 major allergens, while 3.1% and 3.8% of the subjects recognized exclusively group 1 and group 2 allergens, respectively (Table S1). Interestingly, of the 4% of subjects sensitized to a single allergen (Figure 1C),the majority was monosensitized to Der p 23 (2.3% of all subjects, Table S1).

In contrast with previous studies showing relatively low levels of Der p 23 IgEs compared to the other major allergens,[6,13] no significant differences were found between the IgE levels of Der p 23 and group 1 allergens. However, Der p 2 and Der f 2 allergens showed significantly higher IgE levels than Der p 23 ($p < .05$) (Figure 1D).

3.2 | IgE reactivity profiles differ based on geographic location

Participants were recruited from 3 geographic areas (North America [Canada and USA], Europe, South Africa). As shown in Figure 2A and Figure S1, the American and European populations displayed similar IgE prevalence patterns, although the frequencies of sensitization were generally higher in Europe. Der p 23 sensitization was significantly more prevalent in Europe than in North America. The Canadian population had the lowest prevalence of sensitization to group 1 allergens, and to Der p 23 and Der p 7. The South African cohort was the most divergent, although the frequencies were based only on a limited number of subjects ($n = 41$). In South Africans, Der p 23 was the allergen with the highest prevalence of sensitization, both compared to other geographic populations and to other allergens: more than 80% of South Africans had IgE to Der p 23. Also, Der p 7 displayed significantly higher frequencies of sensitization (56%) compared to other geographic populations and should be considered a major allergen in this region.

The country/continent of residence impacted also the concentration of specific IgE in sensitized individuals, as shown in Figure 2B. IgE levels to major allergens were similar between European and South African subjects, and between Canadians and Americans. However, Europeans and South Africans generally had significantly higher levels of IgE than North Americans.

3.3 | HDM-allergic asthmatics have higher sensitization frequencies and higher IgE levels to HDM allergens than non-asthmatics

House dust mite allergy is a major risk factor for asthma, particularly in pediatric populations.[30] Our analysis showed that, compared to non-asthmatics, the asthmatic group had consistently higher frequencies of sensitization to each of the HDM allergens (Figure 3A). On average, allergic subjects with asthma recognized more allergens than non-asthmatic subjects (6.2 vs. 5.3 allergens, respectively) (Figure 3B) and had higher levels of HDM-specific IgE (Figure 3C).

3.4 | Allergen-specific IgE levels are higher in subjects with high HDM exposure

Allergen exposure was determined by measuring group 1 allergen levels in dust collected in participants' homes (only Der p 1 and Der f 1 were analyzed). Figure 4 shows that subjects living in houses with higher HDM allergen levels had on average more HDM allergen-specific IgEs in total as well as more IgEs specific for group 1, Der p 7 and Der p 23 allergens. This seems to suggest that subjects exposed at home to more HDM allergen may be more susceptible to the development of asthma; however, our analysis did not clearly sustain this hypothesis. Although dust samples from houses of asthmatic subjects had, on average, a higher amount of HDM allergens than those of non-asthmatic subjects (3.6 vs. 3.3 µg of Der p 1/f 1 per g of dust), the difference was not statistically significant in our study (data not shown). Households located in different geographic regions had, on average, the same amount of dust levels (Figure S2).

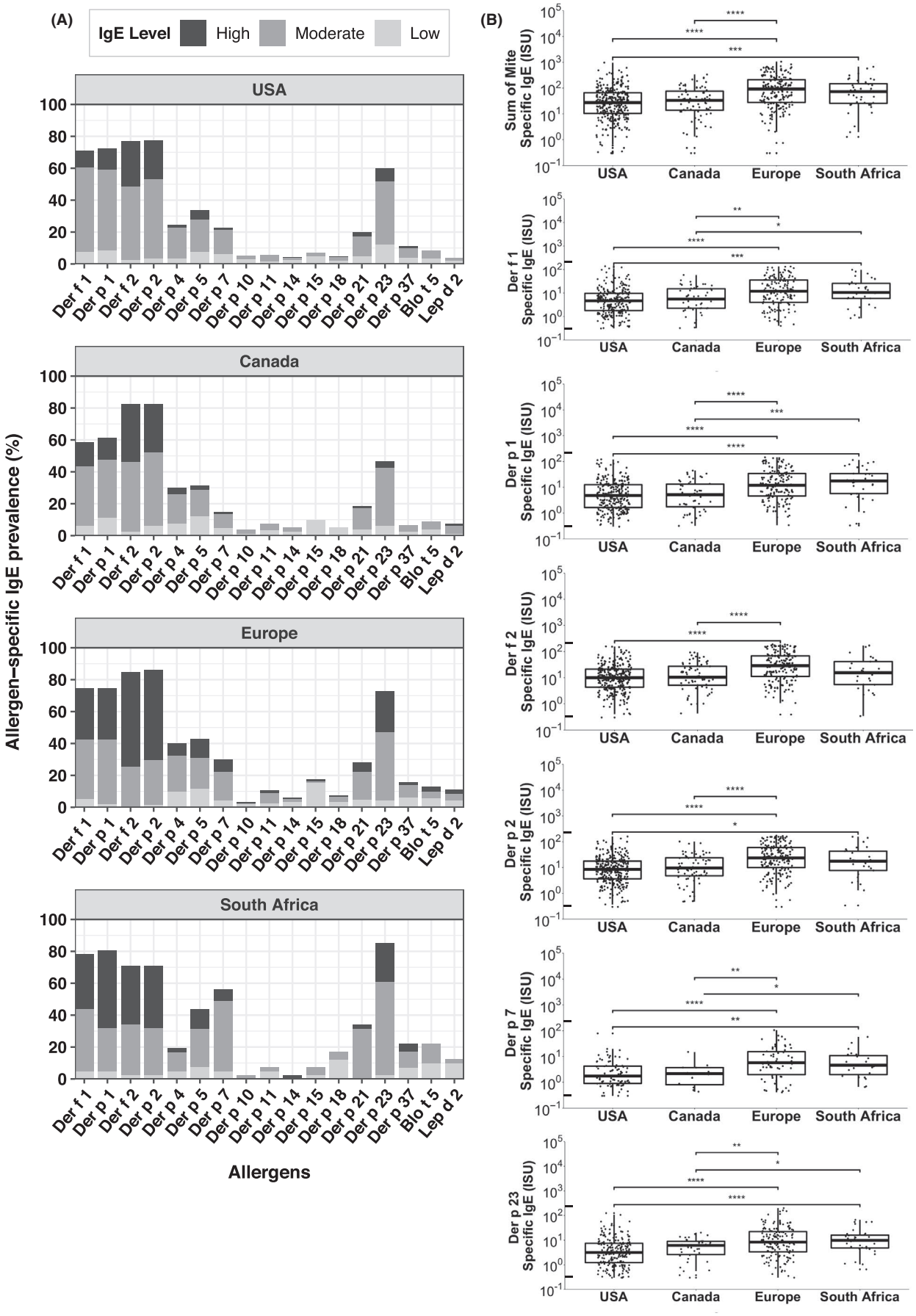

(A)
IgE Level
High
Moderate
Low
USA
Canada
Europe
South Africa
Allergen–specific IgE prevalence (%)
Der f 1
Der p 1
Der f 2
Der p 2
Der p 4
Der p 5
Der p 7
Der p 10
Der p 11
Der p 14
Der p 15
Der p 18
Der p 21
Der p 23
Der p 37
Blo t 5
Lep d 2
Allergens
(B)
Sum of Mite Specific IgE (ISU)
Der f 1 Specific IgE (ISU)
Der p 1 Specific IgE (ISU)
Der f 2 Specific IgE (ISU)
Der p 2 Specific IgE (ISU)
Der p 7 Specific IgE (ISU)
Der p 23 Specific IgE (ISU)
USA
Canada
Europe
South Africa

FIGURE 2 Frequencies of IgE responses to HDM allergens based on geographic location of study population. (A) Prevalence of IgE sensitization to individual HDM allergens in the American (n = 356), Canadian (n = 80), European (n = 208) and South African (n = 41) study populations. The stacked columns show the percentage of individuals with high (ISU≥15, in black), moderate (ISU<15, ≥1, in dark gray) or low (ISU<1, ≥0.3, in light gray) IgE levels. (B) Comparison of HDM-specific IgE levels between the American, Canadian, European and South African study population. Dots indicate individual values, boxes indicate the interquartile ranges, horizontal lines indicate the median values, and whiskers indicate maximum and minimum values, excluding outliers. Ticks on the right of the Y-axis indicate the range of the test (0.3–150 ISU). Only subjects sensitized to the allergen in question were included in the analysis (ISU≥0.3). Statistically significant differences between 2 groups are shown (*$p \le .05$, **$p \le .01$, ***$p \le .001$, ****$p \le .0001$)

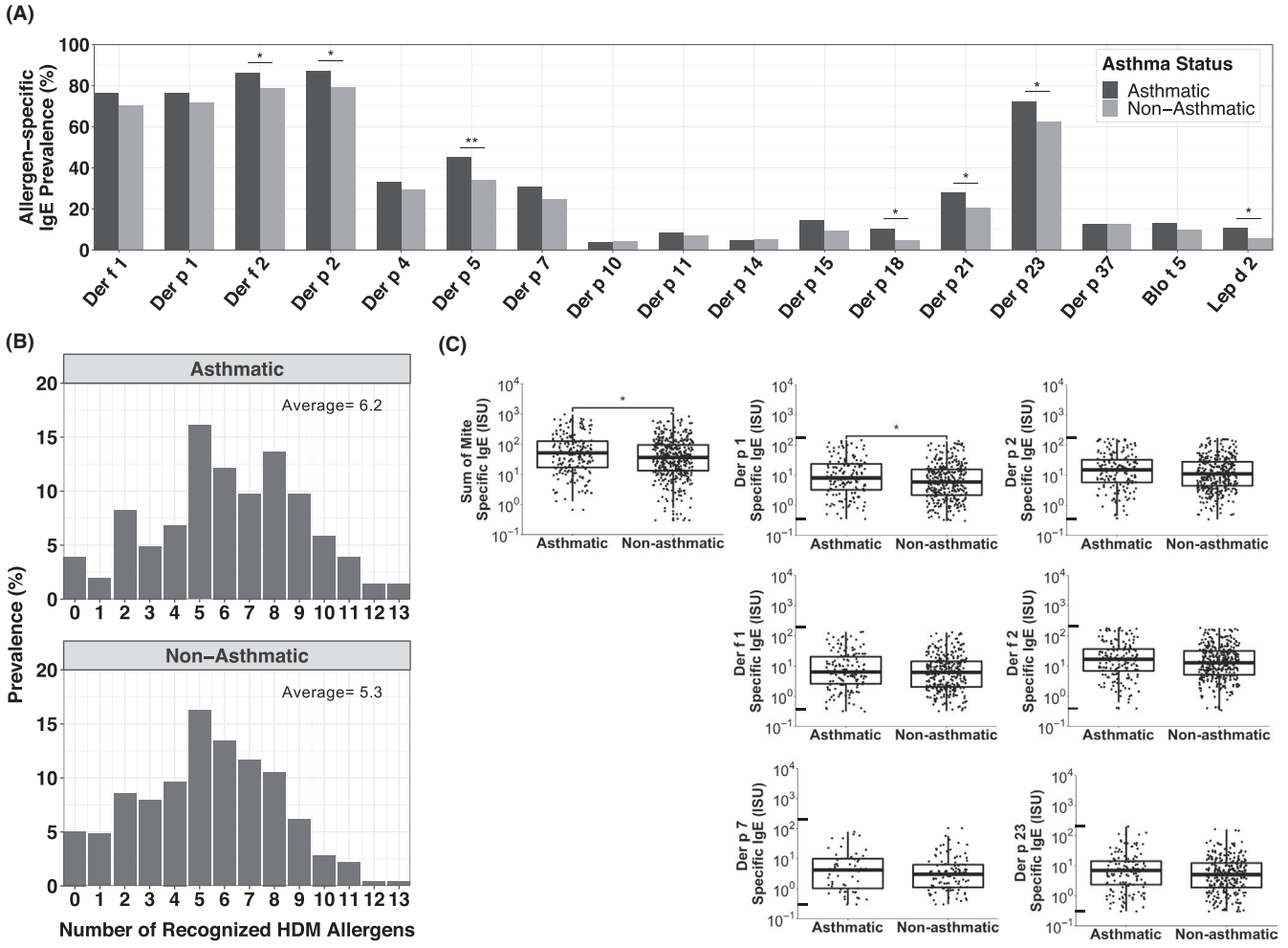


FIGURE 3 Prevalence of IgE responses to HDM allergens based on asthma status. Subjects were defined as asthmatics based on current asthma diagnosis in their medical history. Asthmatic subjects were excluded from the study if they required GINA Step 3 or higher treatment, or experienced a deterioration of asthma that resulted in emergency treatment or hospitalization in the 12 months before randomization, or if they experienced a life-threatening asthma attack at any time in the past. (A) Comparison of allergen-specific IgE sensitization frequencies between asthmatic (n = 197) and non-asthmatic (n = 451) subjects. Statistically significant differences are shown (*$p \le .05$, **$p \le .01$). (B) Prevalence of asthmatic (top panel) or non-asthmatic (bottom panel) subjects with polysensitization to HDM allergens. (C) Comparison of HDM-specific IgE levels between asthmatic and non-asthmatic allergic subjects. Dots indicate individual values, boxes indicate the interquartile ranges, horizontal lines indicate the median values, and whiskers indicate maximum and minimum values, excluding outliers. Ticks on the right of the Y-axis indicate the range of the test (0.3–150 ISU). Only subjects sensitized to the allergen in question were included in the analysis. Statistically significant differences between 2 groups are shown (*$p \le .05$)

3.5 | Other factors influencing sensitization: IgE reactivity profiles differ based on race/ethnicity and levels of HDM-specific IgE are lower in older subjects and in smokers

We investigated whether race/ethnicity, age, and other factors, such as smoking history[31] and BMI,[32] may have an impact on sensitization to HDM allergens. There were no significant differences in the overall levels of specific IgE based on race (Figure 5A). Subjects of different race had similar prevalence patterns; however, some interesting exceptions were observed (Figure 5B): Asian subjects (n = 31) were more likely to be sensitized to group 1 than group 2 allergens; Der p 7 had the lowest prevalence among Asian and Black subjects; Black subjects (n = 70, mostly recruited in the US) had the lowest incidence of sensitization to Der p 23 (Figure 5B).

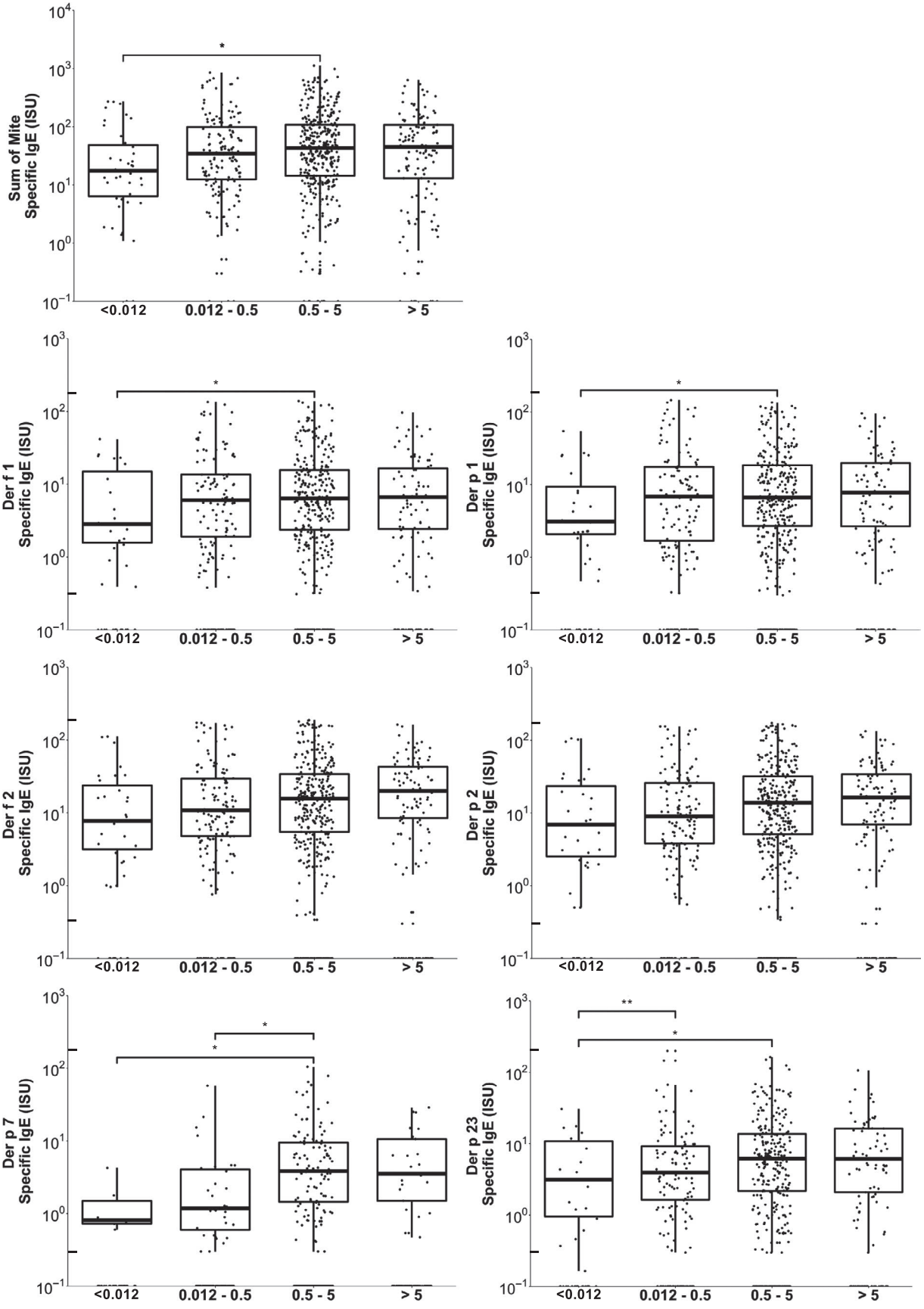

Sum of Mite Specific IgE (ISU)
Der f 1 Specific IgE (ISU)
Der p 1 Specific IgE (ISU)
Der f 2 Specific IgE (ISU)
Der p 2 Specific IgE (ISU)
Der p 7 Specific IgE (ISU)
Der p 23 Specific IgE (ISU)
<0.012
0.012 - 0.5
0.5 - 5
> 5
µg [Der p 1 + f 1] per mg of household dust

FIGURE 4 Comparison of IgE sensitization levels based on HDM exposure as measured by group 1 allergen content in household dust. Dots indicate individual values, boxes indicate the interquartile ranges, horizontal lines indicate the median values, and whiskers indicate maximum and minimum values, excluding outliers. Ticks on the right of the Y-axis indicate the range of the test (0.3–150 ISU). Statistically significant differences between 2 groups are shown (*$p \leq .05$, **$p \leq .01$, ***$p \leq .001$)

While the prevalence patterns did not change with age (Figure S3A), the levels of HDM-specific IgE significantly decreased in older groups (Figure 5C and Figure S3B). Older subjects also recognized fewer HDM allergens (Figure S4).

Surprisingly, subjects with a smoking history (current or former smokers) had slightly lower frequencies of sensitization and, in the case of IgE to Der p 2, Der f 2, and Der p 7 significantly lower levels of IgEs to HDM allergens (Figure 5D and Figure S5). Further investigation is required to discern the cause and the clinical significance of these findings. BMI did not appear to impact sensitivity to HDM allergens. There were no significant differences in the sensitization patterns and IgE levels between underweight, normal weight, overweight and obese subjects (data not shown).

4 | DISCUSSION

Our study, to date, represents the most comprehensive analysis of molecular sensitization profiles to HDM allergen molecules in allergic subjects from North America, Europe, and South Africa. It is also unique because of the inclusion of clinically well-characterized subjects with heterogeneous ethnicity, and the inclusion of data regarding HDM exposure and smoking history. The results obtained broaden the geographical comparisons of dust mite allergen sensitization and identify novel patterns of sensitization in HDM-allergic individuals from South Africa.

Previous studies (for example, Weghofer and colleagues[8]) described Der p 23 as a major allergen, with IgE levels of similar to those specific for group 1 and 2.[6–13] Here, we demonstrate for the first time that, depending on geographical region, sensitization to Der p 23 can even be more prevalent than to the archetypal major group 1 and 2 allergens. In South Africa, more subjects (86%) were sensitized to Der p 23 than to Der p 1 (80%) and Der p 2 (71%). We also demonstrate that Der p 7 can act as a major allergen, with sensitization rates in South Africans approaching 60%. In previous studies of European and North American populations, this figure has been 20–40%.[6,10,11,22] However, Batard and colleagues reported a prevalence of approximately 60% in a small Japanese population.[13] Thus, geography influences the prevalence of sensitization, blurring the nominal boundaries between "major" vs. "minor" allergens. This is important for AIT because efficacy may vary depending on molecular sensitization profiles, and the representation of allergens in the AIT products, as has been recently demonstrated.[24,25] The design of novel molecular AIT approaches will need to incorporate clinically relevant allergens for different populations.[33,34] Furthermore, the results support the idea that the "one size fits all" therapeutic approach used for other allergens may not be ideal for HDM and personalized medicine may be needed to enhance AIT outcomes. Improvement of diagnostic tools to enable the stratification of patients based on their sensitization to major and then minor HDM allergens would be required.

With the exception of subjects from South Africa, the prevalence of IgE responses to the panel of HDM allergens tested in this study was broadly similar to that reported by other groups.[6,10,11,13,20,29,35] Our study also confirms the observation made in several studies,[11,35–37] that asthmatic subjects have a higher level of complexity in the IgE response, with a broader range of allergens being recognized and a higher prevalence of allergen-specific IgE responses.

Posa and colleagues studied the evolution of HDM-specific IgE responses during the first two decades of life.[11] IgE sensitization began after the age of 3 years with responses to one or more of Der p 1, p 2, and p 23 (with 5.9% of their population sensitized to only Der p 1, p 2, and p 23; compared to 7.7% of ours sensitized to Der p 1/f1, p2/f2, and p23), identifying Der p 23 as a major sensitizing allergen, despite its presence in relatively low concentrations in mite feces.[6,8,9,12] The gradual increase in the number of allergens recognized by specific IgE reaches a plateau around 10–20 years of age.[11]

In agreement with the findings of Posa,[11] De Amici and Ciprandi analyzed total and allergen-specific IgE levels in 6,370 allergic individuals, demonstrating an increase in specific IgE until adolescence and subsequent age-dependent decline in *D. pteronyssinus*-specific IgE levels.[38] In the exclusively adult population recruited to our study, we observed an age-dependent decay in specific IgE for group 1, group 2 and Der p 23 allergens, bringing additional granularity to this observation.

The presence of lower allergen-specific IgE levels in older subjects may be caused by a general decrease in IgE levels due to immunosenescence, or may be a consequence of the sensitization history of the individuals. It is also possible that older subjects may have learned to better control HDM exposure and practice better avoidance, assuming that allergen avoidance eventually reduces HDM allergen-specific IgE levels.

Several studies, including our own, have demonstrated a significant proportion of subjects monosensitized to Der p 23. In the current study we observed a monosensitization prevalence of 2.3%. Resch and colleagues saw 3.8%,[10] and Posa and colleagues 5.9%.[11] Thus, several studies confirm Der p 23 as a significant sensitization source in its own right.[39,40]

Previous studies have demonstrated an influence of HDM allergen concentrations in household dust and IgE sensitization in children.[41] Lau and colleagues confirmed the relationship between HDM exposure and Der p 1/Der f 1 IgE sensitization in children and young adults.[42] Sporik and colleagues demonstrated a relationship between levels of HDM allergen exposure and the risk of developing asthma in childhood.[43] Our study demonstrated a significant association between current indoor levels of HDM allergen exposure

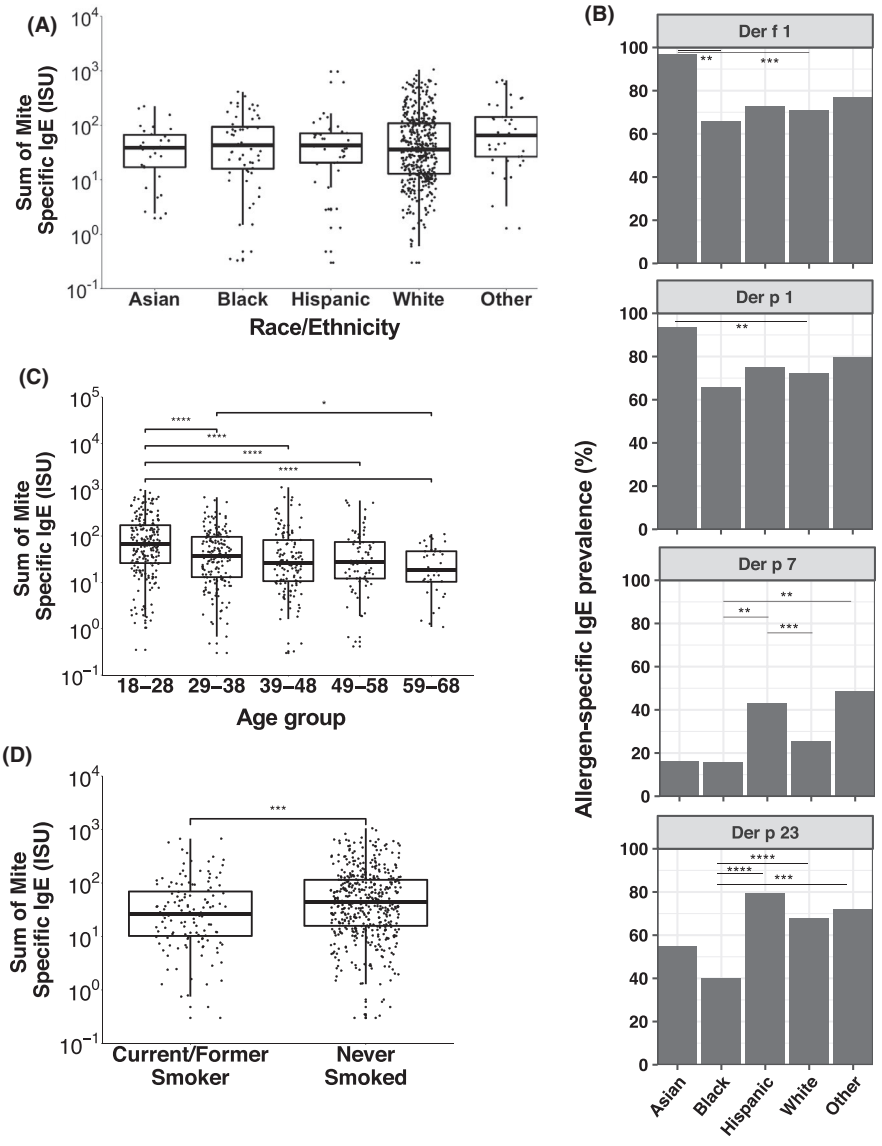


FIGURE 5 Influence of race/ethnicity, age and smoking history on HDM sensitization profiles. (A) Comparison of HDM-specific IgE levels in subjects with Asian (n = 31), Black (n = 70), Hispanic (n = 44), White (n = 463) or other (n = 39) background. (B) Prevalence of HDM allergen-specific IgE between subjects of different race/ethnicity. Only results from group 1, group 2, Der p 7 and Der p 23 allergens are displayed. Differences in the remaining allergens were not statistically significant and were not shown. (C and D) Comparison of HDM-specific IgE levels (C) between subjects aged 18–28 (n = 209), 29–38 (n = 182), 39–48 (n = 138), 49–58 (n = 81) or 59–68 (n = 38) and (D) between subjects that were current or former smokers (n = 141) and subjects that never smoked (n = 507). Smoker status was defined based on current or former consumption of cigarettes, cigars or pipes. Subjects in the smokers group accumulated on average 11 years of smoking (Range: 1–40 years). In (A, C and D), dots indicate individual values, boxes indicate the interquartile ranges, horizontal lines indicate the median values, and whiskers indicate maximum and minimum values, excluding outliers. Only subjects sensitized to at least one allergen were included in the analysis. Statistically significant differences between 2 groups are shown (*$p \leq .05$, **$p \leq .01$, ***$p \leq .001$, ****$p \leq .0001$)

(Der p 1 and Der f 1 are indicative of the presence of Der p and Der f mites, respectively) and specific IgE levels to group 1, group 2 and Der p 23 allergens, despite the fact that our analysis was cross-sectional, rather than longitudinal. This may reflect a scenario in which there is a direct relationship between current exposure levels and IgE levels. The latter has been shown for seasonal allergies where allergen exposure led to boost of systemic allergen-specific IgE production without effects on IgG production.[44] Regarding indoor allergen exposure, this point remains under debate. Allergen exposure can boost protective allergen-specific IgG antibody responses,[45] whereas allergen avoidance can lead to a decrease of allergen-specific IgE levels and hence to reduced clinical sensitivity.[46] A longitudinal analysis of exposure may provide deeper insight into the issue.

It is well established that smoking is associated with higher levels of total IgE.[47,48] However, the relationship between smoking and allergen-specific IgE is less clear. Omenaas and colleagues demonstrated a higher prevalence of specific IgE to HDM in smokers

compared to non-smokers in a Norwegian population (n = 1512, age 18–73 years).[49] In a young (20–44 years) population of adults in the UK, Jarvis and colleagues demonstrated that smokers had higher levels of specific IgE to HDM allergen extract, but lower levels of specific IgE to grass and cat.[31]

A limitation of our study, in common with other studies employing allergen arrays, is that our study results may have been influenced by the relatively low amounts of allergen immobilized on the array chip (relative to standard tests for individual allergens), potentially leading to competition for allergen binding between IgE and IgG isotypes. However, naturally occurring allergen-specific IgG antibodies, unlike those induced by AIT,[50] appear to target epitopes which are different from those recognized by IgE,[51,52] which may mitigate this concern.

The serum samples employed in this study were collected as part of a clinical trial of a peptide-based immunotherapy product. Since larger wheal sizes correlate with clinical allergen reactivity,[53] subjects with SPT wheal size below 5 mm were not eligible for the trial. The exclusion of individuals with weaker sensitizations and of severe asthmatic individuals (requiring ≥GINA Step 3 treatment) represents a potential source of bias in this study.

Although our study analyzed a large number of individuals from three different geographical regions, the overall population was predominantly white and had relatively small numbers of other racial groups. Also, our observations were based on self-reported race-ethnicity, which may poorly reflect the actual genetic ancestry.[54] Differences in local climate and mite species that may have influenced exposure within each geographic area were not taken in consideration and require further investigation.

Taken together, our results suggest that the prevalence of IgE reactivity to HDM allergens is influenced by geography, and that Der p 23 and Der p 7 may play a more dominant role in HDM allergy in South Africa, compared to Europe and North America. For this reason, AIT treatments standardized only on group 1 and 2 allergens could be suboptimal in certain regions/populations, and particularly in the ~10% of individuals that lack IgE for group 1 and 2 allergens. In this context, further standardization of Der p 23 and Der p 7 content in allergen preparations, or the use of defined allergen molecules represents a logical target for the improvement of current HDM diagnostic tools and therapies.

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CONFLICT OF INTEREST

RV reports grants and personal fees from Viravaxx and grants from HVD Biotech, outside the submitted work; JH reports personal fees from Quintiles and Circassia Ltd., during the conduct of the study; ML reports grants and personal fees from Adiga Life Sciences Inc, during the conduct of the study, and grants and personal fees from Circassia Ltd, Circassia Pharmaceuticals PLC, and Aravax Pty, outside the submitted work; in addition, ML is an inventor on 17 patent families held by Circassia Pharmaceuticals PLC and Adiga Life Sciences Ltd, and licensed to them. The rest of the authors declare no conflicts of interest.

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REFERENCES

1. Arlian LG, Platts-Mills TAE. The biology of dust mites and the remediation of mite allergens in allergic disease. *J Allergy Clin Immunol*. 2001;107(3):S406-S413.
2. Calderon MA, Linneberg A, Kleine-Tebbe J, et al. Respiratory allergy caused by house dust mites: what do we really know? *J Allergy Clin Immunol*. 2015;136(1):38-48.
3. WHO/IUIS Allergen Nomenclature Sub-Committee. Allergen nomenclature. http://www.allergen.org. Accessed May 4, 2020.
4. Thomas WR. IgE and T-cell responses to house dust mite allergen components. *Mol Immunol*. 2018;100:120-125.
5. Hales BJ, Martin AC, Pearce LJ, et al. IgE and IgG anti–house dust mite specificities in allergic disease. *J Allergy Clin Immunol*. 2006;118(2):361-367.
6. Mueller GA, Randall TA, Glesner J, et al. Serological, genomic and structural analyses of the major mite allergen Der p 23. *Clin Exp Allergy*. 2016;46(2):365-376.
7. Barber D, Arias J, Boquete M, et al. Analysis of mite allergic patients in a diverse territory by improved diagnostic tools. *Clin Exp Allergy*. 2012;42(7):1129-1138.
8. Weghofer M, Grote M, Resch Y, et al. Identification of Der p 23, a peritrophin-like protein, as a new major dermatophagoides pteronyssinus allergen associated with the peritrophic matrix of mite fecal pellets. *J Immunol*. 2013;190(7):3059-3067.
9. Soh WT, Le Mignon M, Suratannon N, et al. The house dust mite major allergen der p 23 displays O-glycan-independent IgE reactivities but no chitin-binding activity. *Int Arch Allergy Immunol*. 2016;168(3):150-160.
10. Resch Y, Michel S, Kabesch M, Lupinek C, Valenta R, Vrtala S. Different IgE recognition of mite allergen components in asthmatic and non-asthmatic children. *J Allergy Clin Immunol*. 2015;136(4):1083-1091.
11. Posa D, Perna S, Resch Y, et al. Evolution and predictive value of IgE responses toward a comprehensive panel of house dust mite allergens during the first 2 decades of life. *J Allergy Clin Immunol*. 2017;139(2):541.
12. Becker S, Schlederer T, Kramer MF, et al. Real-life study for the diagnosis of house dust mite allergy - the value of recombinant allergen-based IgE serology. *Int Arch Allergy Immunol*. 2016;170(2):132-137.
13. Batard T, Baron-Bodo V, Martelet A, et al. Patterns of IgE sensitization in house dust mite-allergic patients: implications for allergen immunotherapy. *Allergy* 2015;71(2):220-229.
14. Thomas WR. House dust mite allergens: new discoveries and relevance to the allergic patient. *Curr Allergy Asthma Rep*. 2016;16(9):69.
15. Partridge MR, van der Molen T, Myrseth S-E, Busse WW. Attitudes and actions of asthma patients on regular maintenance therapy: the INSPIRE study. *BMC Pul Med*. 2006;6(1):13.
16. Calderon MA, Bousquet J, Canonica GW, et al. Guideline recommendations on the use of allergen immunotherapy in house dust mite allergy: time for a change? *J Allergy Clin Immunol*. 2017;140(1):41-52.

17. Agache I, Lau S, Akdis CA, et al. EAACIGuidelines on allergen immunotherapy: house dust mite-driven allergic asthma. *Allergy*. 2019;74(5):855-873.
18. Demoly P, Emminger W, Rehm D, Backer V, Tommerup L, Kleine-Tebbe J. Effective treatment of house dust mite–induced allergic rhinitis with 2 doses of the SQ HDM SLIT-tablet: results from a randomized, double-blind, placebo-controlled phase III trial. *J Allergy Clin Immunol*. 2016;137(2):444.
19. Virchow JC, Backer V, Kuna P, et al. Efficacy of a house dust mite sublingual allergen immunotherapy tablet in adults with allergic asthma: a randomized clinical trial. *JAMA*. 2016;315(16):1715-1725.
20. Huang F-L, Liao E-C, Yu S-J. House dust mite allergy: its innate immune response and immunotherapy. *Immunobiology*. 2018;223(3):300-302.
21. Casset A, Mari A, Purohit A, et al. Varyingallergen composition and content affects the in vivo allergenic activity of commercial dermatophagoides pteronyssinus extracts. *Int Arch Allergy Immunol*. 2012;159(3):253-262.
22. Brunetto B, Tinghino R, Braschi MC, Antonicelli L, Pini C, Iacovacci P. Characterization and comparison of commercially available mite extracts for in vivodiagnosis. *Allergy*. 2010;65(2):184-190.
23. Meyer CH, Bond JF, Chen MS, Kasaian MT. Comparison of the levels of the major allergens Der pI and Der pII in standardized extracts of the house dust mite, Dermatophagoides pteronyssinus. *Clin Exp Allergy*. 1994;24(11):1041-1048.
24. Chen KW, Zieglmayer P, Zieglmayer R, et al. Selection of house dust mite-allergic patients by molecular diagnosis may enhance success of specific immunotherapy. *J Allergy Clin Immunol*. 2019;143(3):1248.
25. Rodríguez-Domínguez A, Berings M, Rohrbach A, et al. Molecular profiling of allergen-specific antibody responses may enhance success of specific immunotherapy. *J Allergy Clin Immunol*. 2020;146(5):1097-1108.
26. Lupinek C, Wollmann E, Baar A, et al. Advances in allergen-microarray technology for diagnosis and monitoring of allergy: the MeDALL allergen-chip. *Methods*. 2014;66(1):106-119.
27. Earle CD, King EM, Tsay A, et al. High-throughput fluorescent multiplex array for indoor allergen exposure assessment. *J Allergy Clin Immunol*. 2007;119(2):428-433.
28. King EM, Filep S, Smith B, et al. A multi-center ring trial of allergen analysis using fluorescent multiplex array technology. *J Immunol Methods*. 2013;387(1):89-95.
29. Weghofer M, Thomas WR, Kronqvist M, et al. Variability of IgE reactivity profiles among European mite allergic patients. *Eur J Clin Invest*. 2008;38(12):959-965.
30. Platts-Mills T, De Weck A, Aalberse R, et al. Dust mite allergens and asthma—A worldwide problem. *J Allergy Clin Immunol*. 1989;83(2):416-427.
31. Jarvis D, Luczynska C, Chinn S, Burney P. The association of age, gender and smoking with total IgE and specific IgE. *Clin Exp Allergy*. 1995;25(11):1083-1091.
32. Hjellvik V, Tverdal A, Furu K. Body mass index as predictor for asthma: a cohort study of 118,723 males and females. *Eur Respir J*. 2010;35(6):1235.
33. Zhernov Y, Curin M, Khaitov M, Karaulov A, Valenta R. Recombinant allergens for immunotherapy: state of the art. *CurrOpin Allergy Clin Immunol*. 2019;19(4):402-414.
34. Dorofeeva Y, Shilovskiy I, Tulaeva I, et al. Past, presence, and future of allergen immunotherapy vaccines. *Allergy*. 2020;9(4):1.
35. Celi G, Brusca I, Scala E, et al. House dust mite allergy in Italy—Diagnostic and clinical relevance of Der p 23 (and of minor allergens): a real-life, multicenter study. *Allergy*. 2019;74(9):1787-1789.
36. Custovic A, Sonntag H-J, Buchan IE, Belgrave D, Simpson A, Prosperi MCF. Evolution pathways of IgE responses to grass and mite allergens throughout childhood. *J Allergy Clin Immunol*. 2015;136(6):1645-1648.
37. Pomes A, Glesner J, Calatroni A, et al. Cockroach allergen component analysis of children with or without asthma and rhinitis in an inner-city birth cohort. *J Allergy Clin Immunol*. 2019;144(4):935-944.
38. De Amici M, Ciprandi G. The age impact on serum total and allergen-specific IgE. *Allergy Asthma Immunol Res*. 2013;5(3):170-174.
39. Matos Semedo F, Dorofeeva Y, Pires AP, et al. Der p 23: clinical relevance of molecular monosensitization in house dust mite allergy. *J InvestigAllergol Clin Immunol*. 2019;29(4):314-316.
40. Jiménez-Feijoo R, Pascal M, Moya R, et al. Molecular diagnosis in house dust mite allergic patients suggests clinical relevance of Der p 23 in asthmatic children. *J InvestigAllergol Clin Immunol*. 2019;30(4):127-132.
41. Rowntree S, Cogswell JJ, Platts-Mills TA, Mitchell EB. Development of IgE and IgG antibodies to food and inhalant allergens in children at risk of allergic disease. *Arch Dis Child*. 1985;60(8):727.
42. Lau S, Falkenhorst G, Weber A, et al. High mite-allergen exposure increases the risk of sensitization in atopic children and young adults. *J Allergy Clin Immunol*. 1989;84(5):718-725.
43. Sporik R, Holgate ST, Platts-Mills TAE, Cogswell JJ. Exposure to house-dust mite allergen (Der pI) and the development of asthma in childhood. *N Engl J Med*. 1990;323(8):502-507.
44. Niederberger V, Ring J, Rakoski J, et al. Antigens drive memory IgE responses in human allergy via the nasal mucosa. *Int Arch Allergy Immunol*. 2007;142(2):133-144.
45. Platts-Mills T, Vaughan J, Squillace S, Woodfolk J, Sporik R. Sensitisation, asthma, and a modified Th2 response in children exposed to cat allergen: a population-based cross-sectional study. *Lancet*. 2001;357(9258):752-756.
46. Almqvist C. High allergen exposure as a risk factor for asthma and allergic disease. *Clinic Rev Allerg Immunol*. 2005;28(1):25-41.
47. Burrows B, Halonen M, Barbee RA, Lebowitz MD. The relationship of serum immunoglobulin E to cigarette smoking. *Am Rev Respir Dis*. 1981;124(5):523-525.
48. Warren CPW, Holford-Strevens V, Wong C, Manfreda J. The relationship between smoking and total immunoglobulin E levels. *J Allergy Clin Immunol*. 1982;69(4):370-375.
49. Omenaas E, Bakke P, Elsayed S, Hanoa R, Gulsvik A. Total and specific serum IgE levels in adults: relationship to sex, age and environmental factors. *Clin Exp Allergy*. 1994;24(6):530-539.
50. Wollmann E, Lupinek C, Kundi M, Selb R, Niederberger V, Valenta R. Reduction in allergen-specific IgE binding as measured by microarray: a possible surrogate marker for effects of specific immunotherapy. *J Allergy Clin Immunol*. 2015;136(3):806-807.
51. Curin M, Swoboda I, Wollmann E, et al. Microarrayed dog, cat, and horse allergens show weak correlation between allergen-specific IgE and IgG responses. *J Allergy Clin Immunol*. 2014;133(3):918.
52. Siroux V, Lupinek C, Resch Y, et al. Specific IgE and IgG measured by the MeDALL allergen-chip depend on allergen and route of exposure: the EGEA study. *J Allergy Clin Immunol*. 2017;139(2):643-646.
53. Kanthawatana S, Maturim W, Fooanan S, Trakultivakorn M. Skin prick reaction and nasal provocation response in diagnosis of nasal allergy to the house dust mite. *Ann Allergy Asthma Immunol*. 1997;79(5):427-430.
54. Mersha TB, Abebe T. Self-reported race/ethnicity in the age of genomic research: its potential impact on understanding health disparities. *Hum Genomics*. 2015;9(1):1.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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