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ORIGINAL ARTICLE AIRWAY DISEASES

Allergic airway diseases in a tropical urban environment are driven by dominant mono-specific sensitization against house dust mites

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Abstract

Background: Southeast Asian populations are increasingly affected by allergic airway diseases. Etiology and specific causes, however, are still unknown. The aim of this study is therefore to identify allergens and risk factors for the high prevalence of allergic airway disease in the tropical urban environment.
Methods: Symptoms of allergic rhinitis (AR), asthma, and allergic dermatitis were recorded in two independent cohorts of 576 and 7373 ethnic Chinese individuals living in Singapore. Reactivity against common allergens was determined by skin prick tests (SPT); specific immunoglobulin E (sIgE) titers against 12 common allergens, as well as total serum IgE (tIgE), were measured in the smaller cohort.
Results: Immunoglobulin E sensitization was almost exclusively directed against house dust mite (HDM) allergens. More than 80% of individuals were HDM-sIgE positive. Of these, less than 30% also had sIgE for other allergens, and similarly, few of the HDM-sIgE-negative individuals reacted to other allergens. Titers for HDM-sIgE were 8–30 times higher than other non-HDM allergen titers and correlated directly with total serum tIgE levels. Migrants from nontropical countries typically arrived with low or undetectable HDM-sIgE but developed substantial titers in a time-dependent fashion. Importantly, prolonged stay in Singapore also resulted in the manifestation of AR and asthma symptoms, contributing to some of the highest national prevalence rates worldwide.
Conclusion: In a tropical urban environment, the allergic response is dominated by a single allergen class. The mono-specific IgE sensitization against HDM translates into increased prevalence of allergic airway diseases, which now impact a large proportion of the population in Singapore.

The prevalence of allergic airway phenotypes has increased dramatically in most countries in recent decades. By 2025, an estimated 400 million people will suffer from asthma, and nearly 500 million will be affected by allergic rhinitis (AR) (1, 2). These allergic conditions often exist as co-morbidities, with close to two-thirds of asthmatics concurrently displaying AR symptoms. Interestingly, marked geographic differences are evident in the prevalence of allergic disease, with westernized and developed cities suffering most. The factors underlying these variations in the prevalence of allergic disease, however, are still largely unknown.

The primary pathological process in the development of allergic disease is production of immunoglobulin E (IgE). Studies from Europe and North America revealed a heterogeneous set of allergic compounds able to drive IgE production, including pollens, pet dander, fungus, molds, house dust mites, and many others (3–5). While IgE is clearly the mediator, the complexity of the allergen-set usually results in a

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poor correlation between the allergen-specific IgE (sIgE) titer and the total serum IgE (tIgE) (6). As a consequence, levels of tIgE do not necessarily correlate here with allergic symptoms, and hence, the relevance of tIgE as a risk factor for asthma has been brought into question (4, 7).

While allergies have traditionally been associated with industrial countries, they are also endemic in the developing world. Recent reports indicate that in the Asia-Pacific region, allergic disease prevalence has reached the highest levels in 50 years (8), a trend also observed in tropical Southeast Asia (9, 10). Although a similar set of allergens to those eliciting responses from populations in western regions has been proposed (9, 11–14), the course, specificity, and complexity of the allergic response in tropical countries may differ substantially from that in temperate zones. Therefore, the objective of this study was to identify allergens and risk factors for the high prevalence of allergic airway disease in the tropical urban environment.

Methods

Ethics statement

This study is in compliance with the Helsinki declaration and has been performed with the approval of the Institutional Review Board of the National University of Singapore (IRB reference – NUS07-023 and NUS10-343). Samples used in this study were collected from ethnic Chinese participants following written informed consent.

Study populations

The two independent prospective cohorts were described below (discovery and validation). Ethnic Chinese subjects were recruited at the National University of Singapore as a part of an ongoing epidemiological collection for the study of allergic diseases. Volunteers were then classified as individuals with AR, with asthma, and healthy controls using a questionnaire collecting information on demographics and medical history, and based on the Allergic Rhinitis Impact on Asthma (ARIA) (2, 15) and International Study of Asthma and Allergies in Childhood (ISAAC) guidelines. The volunteers also underwent a skin prick test (SPT) using a panel of 4 allergens common in Singapore (Dermatophagoides pteronyssinus, Blomia tropicalis, Elaeis guineensis, and Curvularia lunata). The initial discovery cohort consists of a total of 576 ethnic Chinese volunteers. Blood was collected for measurement of total and allergen-specific IgE levels against 12 common allergens. For validation, we used an independent prospective cohort of 7373 ethnic Chinese individuals similarly recruited. As in the discovery cohort, the study subjects filled a questionnaire and underwent SPT against the four allergens listed above. The demographics of these study populations are summarized in Table 1.

Allergy testing

IgE levels measured in the discovery cohort

Levels of total immunoglobulin E (tIgE) and specific IgE (sIgE) against Dermatophagoides pteronyssinus (DP), Blomia tropicalis (BT), Aspergillus, Acacia, Cladosporum herbarium, Mugwort, Alternaria, Common ragweed, Bermuda grass, Dog dander, Cat dander, and German cockroach were measured using the USFDA-approved ImmunoCAP system (Phadia AB, Uppsala, Sweden).

Skin prick testing (SPT)

The volunteers were subjected to a SPT using a panel consisting of common allergens in Singapore such as DP (house dust mite), BT (house dust mite), Elaeis guineensis (pollen), and Curvularia lunata (fungi). A SPT response is considered positive when the wheal diameter is 3 mm or greater, when compared to positive (histamine) and negative (saline) controls.

Table 1 Demographics of the two study populations (Discovery cohort and Validation cohort)

Country of birth	Gender	Discovery cohort		Validation cohort	
		Count	Age (years), Mean (range)	Count	Age (years), Mean (range)
Singapore	F	119	20.81 (18.00–50.00)	3136	21.00 (9.00–59.00)
	M	176	22.19 (19.00–42.00)	2382	22.55 (17.00–65.00)
	Unknown	11	20.82 (19.00–25.00)	4	19.75 (19.00–21.00)
	Total	360	21.60 (18.00–50.00)	5522	21.67 (9.00–65.00)
China	F	31	25.42 (19.00–55.00)	546	21.92 (17.00–46.00)
	M	35	27.03 (20.00–52.00)	318	22.83 (18.00–56.00)
	Unknown			4	21.25 (21.00–22.00)
	Total	75	26.27 (19.00–55.00)	868	22.25 (17.00–56.00)
Malaysia	F	52	21.88 (18.00–31.00)	541	21.88 (18.00–57.00)
	M	67	21.04 (18.00–35.00)	441	21.90 (18.00–56.00)
	Unknown	11	22.36 (20.00–31.00)	1	21.00 (21.00–21.00)
	Total	141	21.49 (18.00–35.00)	983	21.89 (18.00–57.00)
Total	F	202	21.79 (18.00–55.00)	4223	21.24 (9.00–59.00)
	M	278	22.52 (18.00–52.00)	3141	22.49 (17.00–65.00)
	Unknown	22	21.59 (19.00–31.00)	9	20.56 (19.00–22.00)
	Total	576	22.19 (18.00–55.00)	7373	21.77 (9.00–65.00)

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Statistical methods

All data used in the analysis were processed using Accelrys Pipeline Pilot (Accelrys, San Diego, CA, USA) with statistical analysis in the R statistical language (version 2.12.1). *P*-values less than 0.05 were considered statistically significant. A one-way ANOVA using logarithm-transformed IgE levels to ensure normality was used to determine whether the levels of specific IgE were significantly different for Singaporean Chinese. A Welch's *t*-test was used to compare the logarithm-transformed total IgE levels of atopics and nonatopics. Pearson correlation was used to compare the logarithm-transformed BT, DP, and total IgE levels in a pairwise fashion. One-way ANOVAS were used to determine whether BT, DP-specific, and total logarithm-transformed IgE levels were statistically different between countries of birth. Subjects born in Malaysia and China were divided into three groups by the number of years of residence in Singapore (3 years or less, 3–8 years, and over 8 years). One-way ANOVAS were used to determine whether BT, DP, and total logarithm-transformed IgE levels were statistically different between groups of subjects born in China according to years of residence in Singapore. Fisher's exact tests were used separately for Chinese and Malaysians to determine whether there were any associations between the groups and either house dust mite (HDM) sensitization or allergic phenotypes.

Results

Dominance of HDM-sIgE

To determine the allergen sensitization profile, 206 individuals born in Singapore were screened for responses to the 12 most common allergens reported to be present in the tropical environment. The sIgE titers against these allergens were determined using the ImmunoCAP system (Fig. 1). Sensitization to HDM allergens was markedly strongest and most common, with more than 70% of individuals possessing measurable titers against extracts from either one of the two HDM species *Dermatophagoides pteronyssinus* (DP) or *Blomia tropicalis* (BT). In most cases, sIgE against both mites was detected. Less than 4% of the HDM-positive individuals reacted exclusively to only one of the HDM species (Fig. S1A). Among the other allergens tested, a substantial sensitization rate was evident only for German cockroach (14.56%) and Bermuda grass (6.8%). sIgE titers to other allergens were detected only sporadically, that is, in less than 3% of the study subjects. Their median sIgE titers were generally low. In comparison, the titers of HDM-sIgE were 8- to 30-fold higher than the non-HDM-specific IgE.

Presence of HDM-sIgE alone is indicative of 'atopy'

'Atopy' is a genetic predisposition toward the development of immediate hypersensitivity reactions against environmental allergens (16). Experimentally, atopy is defined by the presence of allergen-specific IgE or positive SPT to common allergens. In western countries, this test requires a set of four or more allergens, which typically reveals prevalence rates between 25% and 45% (6). The Singapore cohort showed a far higher rate. Based on the HDM response, 71% could be defined here as 'atopic' (Fig. 2A). While more than a quarter of the HDM-reactive individuals were sensitized also against other allergens, HDM-sIgE-negative individuals were also mostly negative for other sIgE (Fig. 2B). Thus, HDM sensitization alone could separate 'atopic' from 'nonatopic' individuals.

Direct correlation between serum HDM-sIgE and tIgE levels

While for western countries, only weak associations to the total IgE was reported (6), striking differences became apparent when an extended cohort of 360 Singaporean Chinese was grouped according to their atopy status (Fig. 2C). The median tIgE titer of HDM-sensitized individuals was more than eight times higher than in nonsensitized individuals (195.0 *vs* 23.5 U/ml, *P*-value of 6.55×10^{-16}). Moreover, in a direct plot of *HDM-sIgE vs tIgE*, the levels of HDM-sIgE strongly correlated with tIgE (Fig. 2D, Fig. S1B,C). Thus, high serum tIgE titers are here directly indicative of HDM sensitization.

Extended exposure to the tropical urban environment drives the HDM-sIgE response and HDM sensitization

To evaluate whether the dominance of HDM-sIgE was an environmental phenomenon, the sIgE titers of Singapore-born Chinese were compared to the titers of migrant Chinese who arrived in Singapore either from other tropical or non-tropical countries. For this, HDM-sIgE titers were determined in the extended discovery cohort of 576 individuals, which, in addition to Singapore-born Chinese, also included Singapore residents who had migrated from neighboring Malaysia or China. While the fraction of HDM-sIgE-positive individuals was around 80% for both Singapore-born and Malaysia-born individuals, less than 50% of the migrants from Mainland China tested positive (Fig. 3A). This difference was also reflected in the serum tIgE levels. Comparably high levels were measured in Singapore- and Malaysia-born individuals (median 151 *vs* 129 U/ml), whereas individuals from Mainland China had substantially lower tIgE titers (median 64 U/ml; Fig. 3B).

Marked differences were similarly evident in the magnitude of the HDM-specific response (Fig. 3C, Fig. S2A). While most Singapore- and Malaysia-born individuals had high levels of HDM-sIgE (16.2 *vs* 13.8 U/ml), the majority of migrants from China were negative. This discrepancy was even more evident when we evaluated the sensitization based on the number of years the migrants had spent in Singapore (Fig. 3D, Fig. S2B). While for Malaysia-born individuals the move to Singapore was without any effect on the HDM-sIgE titer (Fig. 3E), Mainland Chinese were strongly affected by the duration of stay in Singapore (Fig. 3D). After 0–3 years in Singapore, less than 30% of China-born individuals were HDM-sIgE positive. The number increased to 50% for the 3–8 years group and reached 60% in the long-term residents (>8 years).

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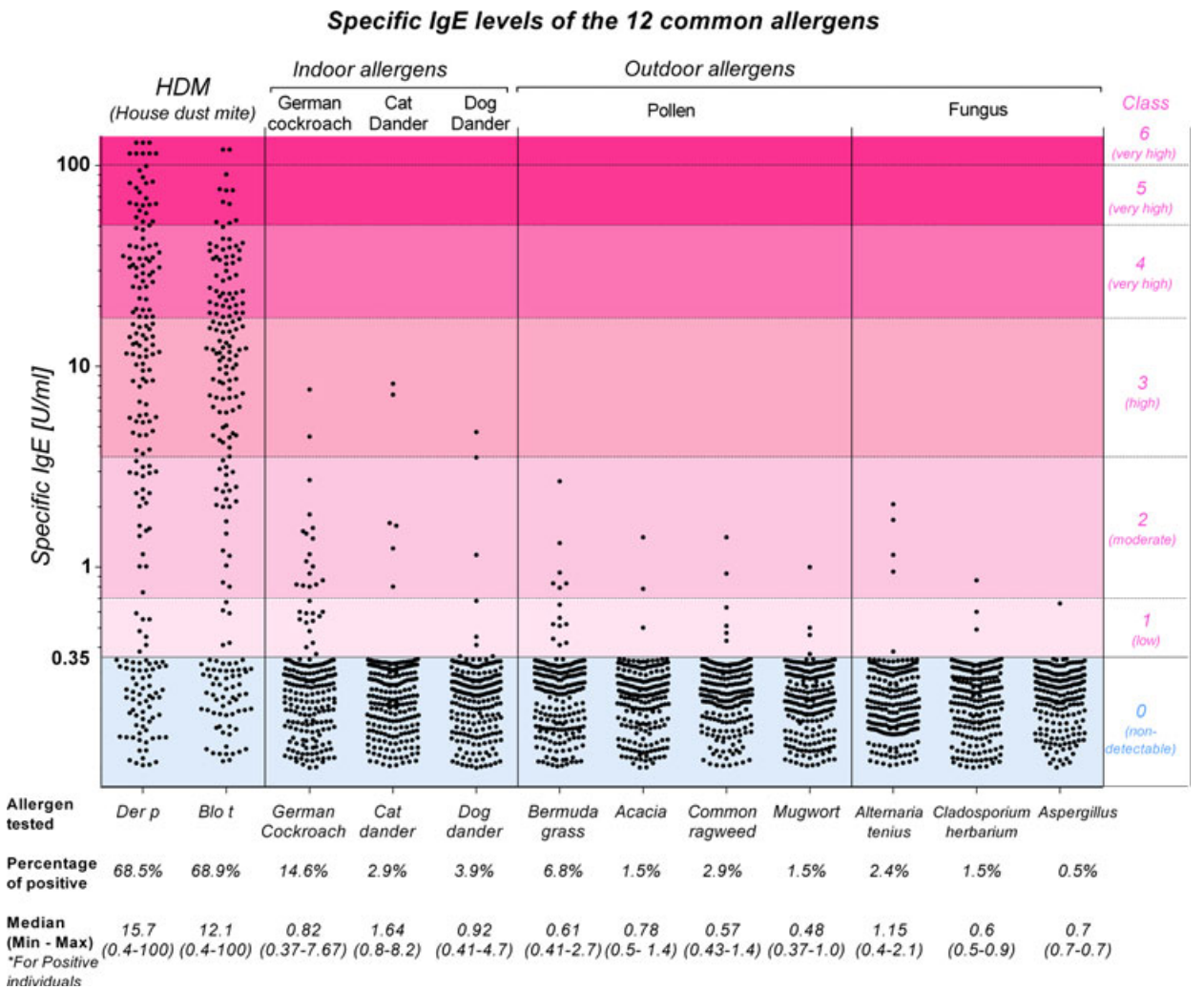

Figure 1 Levels of specific immunoglobulin E (IgE) against the 12 common allergens tested in a cohort of 206 Chinese individuals born in Singapore. Allergens are grouped into indoor allergens (house dust mite, pet dander, and cockroach) and outdoor allergens (fungus and pollens). Specific immunoglobulin E (sIgE) titers are expressed as U/ml. The strength of IgE reactivity toward the specific allergens tested is categorized in classes from 0 to 6 according to ImmunoCAP standards. Titers < 0.35 U/ml were considered negative. The species of the allergen source is listed below together with the percentage of individuals positive for sIgE to each allergen tested. The median sIgE titer including minimum (min) and maximum (max) is calculated for reactive individuals. *Dermatophagoides pteronyssinus* (Der p) or *Blomia tropicalis* (Blo t).

These data were further validated in a prospective study employing a large cohort of 7373 ethnic Chinese, where SPT was used to determine the prevalence of HDM sensitization. Of these, 5522 were born in Singapore, 983 migrated from Malaysia, and 868 from mainland China (Table 1). 70% of the Singapore-born as well as the Malaysia-born Chinese were HDM-SPT positive, while less than 30% of the migrants from Mainland China reacted against HDM (Fig. 4A), and this fraction dropped to less than 20% when considering the Chinese migrants in Singapore for less than 3 years (Fig. 4B, upper left panel). The HDM reactivity of China-born migrants increased over time, reaching nearly 50% in the group of migrants who had lived more than 8 years in Singapore (Fig. 4B, upper left panel). No significant difference was observed between Singapore-born individuals and migrants from neighboring Malaysia (Fig. 4A, Fig. S3B).

Thus, longer exposure time directly correlates with an increase in HDM-sIgE levels and HDM sensitization.

HDM sensitization translates into increased risk of allergic airway diseases

Statistical analysis confirmed that HDM reactivity, defined either by the presence of HDM-sIgE, tIgE, or a positive HDM-SPT, was significantly associated with the frequency of self-reported AR and asthma (Tables S1 and S2).

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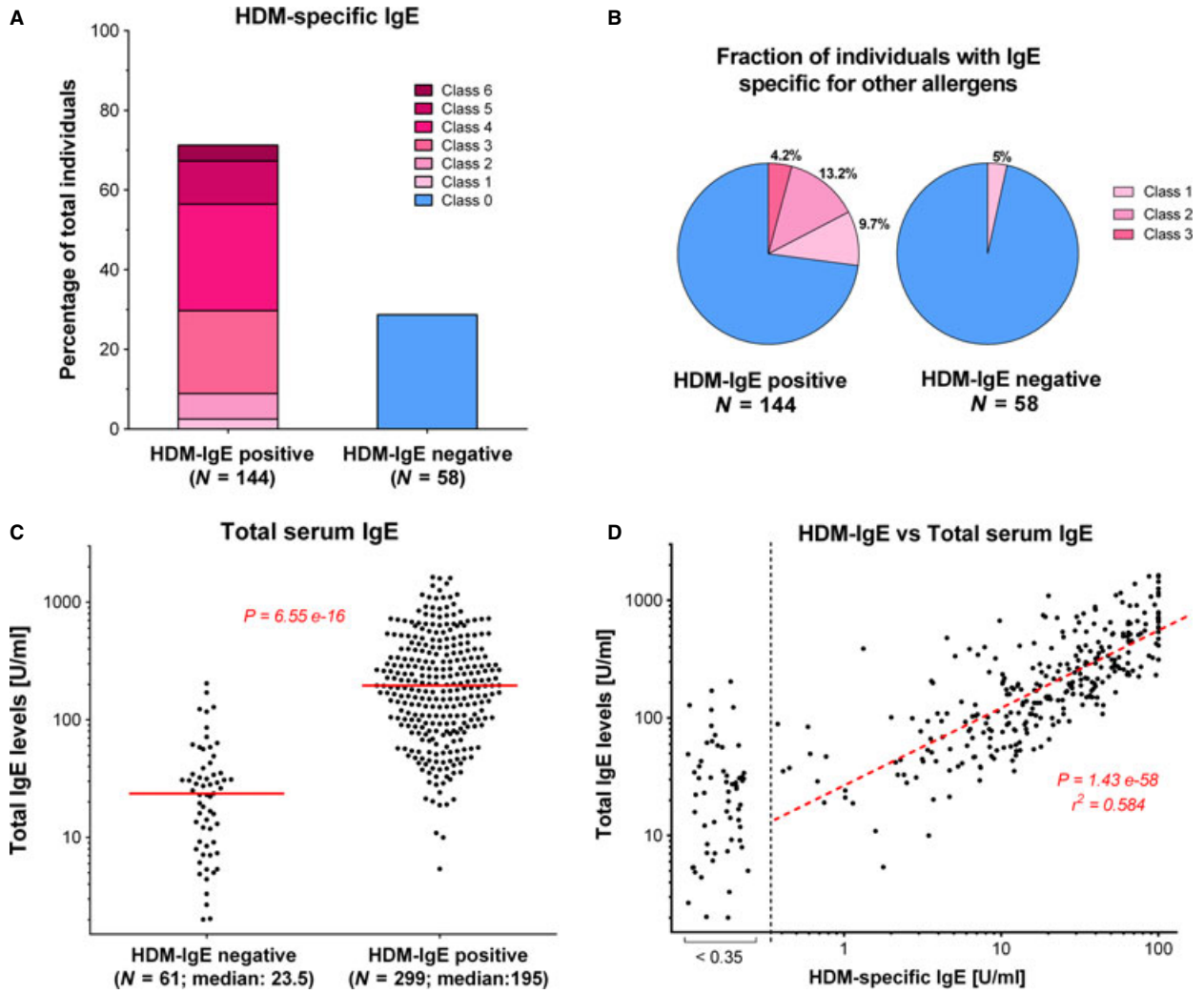

Figure 2 Association of house dust mite (HDM) sensitization with 'atopy' and serum tIgE. Data shown are for Chinese individuals born in Singapore. (A) Percentage of HDM-specific immunoglobulin E (sIgE)-positive and immunoglobulin E (sIgE)-negative individuals. Distribution of individuals is shown based on sIgE titers specific for HDM. Color code refers to the sIgE titer class shown in Fig. 1. Individuals with sIgE titer < 0.35 U/ml were defined as HDM-sIgE negative. (B) Fraction of individuals with sIgE against non-HDM allergens among either HDM-sIgE-positive or HDM-sIgE-negative individuals. The relative fraction is shown for the two groups defined in Fig. 2A. The class of sIgE titer for non-HDM allergens is indicated by the color code. (C) Serum tIgE levels of HDM-sIgE-positive and HDM-sIgE-negative individuals in an extended cohort of 360 Singapore-born Chinese. Serum tIgE was determined by ImmunoCAP ELISA and plotted for HDM-sIgE-negative and HDM-sIgE-positive individuals (Median value indicated). Using a *t*-test with Welch correction, total immunoglobulin E (IgE) was found to be significantly different between the two groups ($P = 6.55 \times 10^{-16}$) (D) Correlation of tIgE levels with HDM-sIgE levels. The titers of HDM-sIgE were plotted against the serum tIgE. P and r^2 values for the linear regression are indicated.

Definitions of AR, asthma, and allergic dermatitis are detailed in the Supplementary Information. The percentage of individuals with symptoms of AR, as determined by questionnaire, proportionately reflected the HDM reactivity determined by SPT (Fig. 4B, upper panel). For both Singapore- and Malaysia-born Chinese, the >70% sensitization rate translates into an AR rate of more than 40% (Fig. S3). In comparison, migrants from China who spent 0–3 years in Singapore have an AR prevalence of only 9% (Fig. 4B). In line with increasing HDM reactivity, the AR prevalence climbs to 22% after over 8 years' exposure to the tropical urban environment (Fig. 4B). The same trend is also observed for asthma, albeit to a lesser degree (lower left panel), but not for allergic dermatitis (AD; lower right panel). Notably, migrants born in Malaysia did not show this trend. HDM sensitization and disease incidence were in the same range as for the Singaporean-born cohort (Fig. S3).

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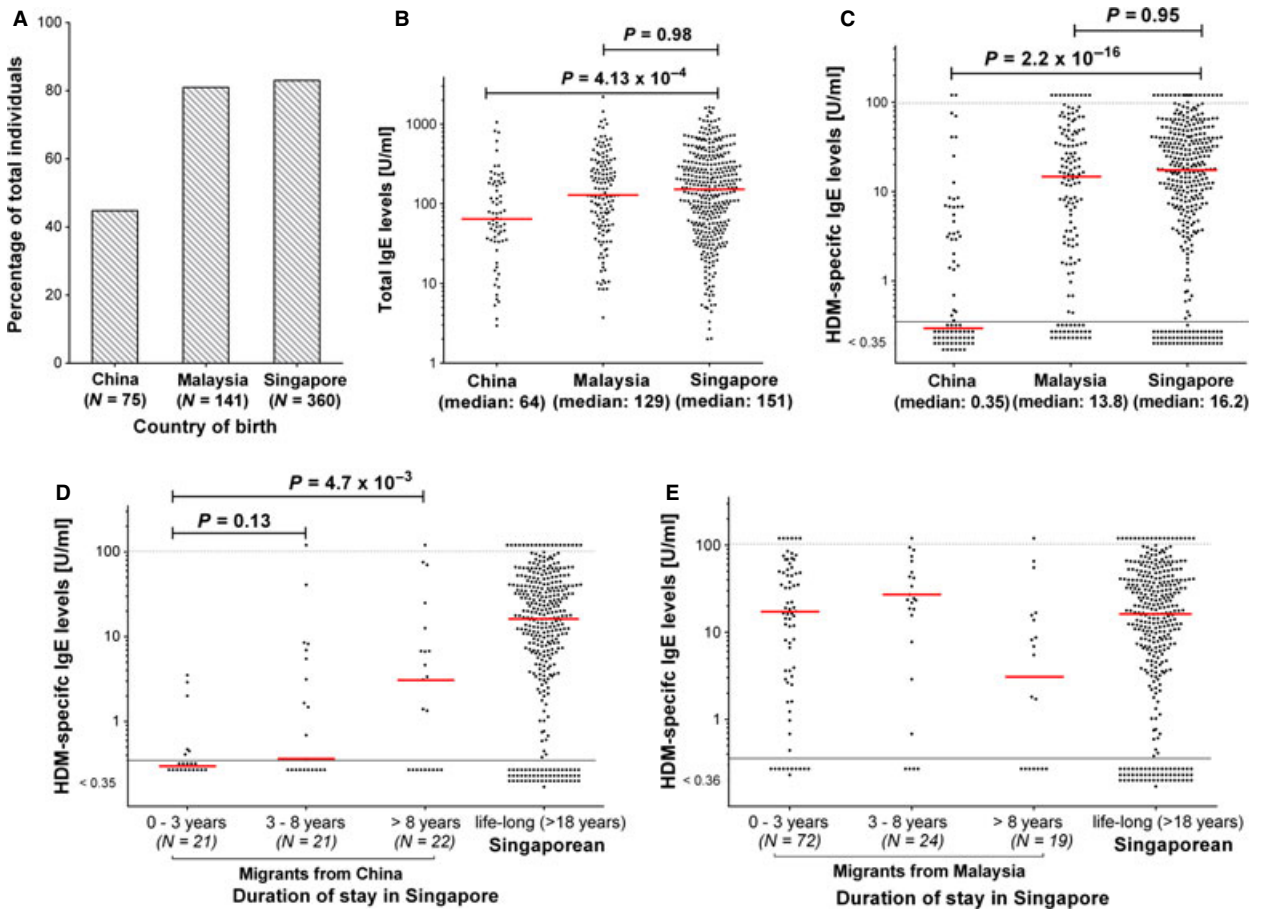

Figure 3 Influence of time of exposure to tropical urban environment on house dust mite (HDM) sensitization within the discovery cohort (Table 1). (A) Percentage of HDM-specific immunoglobulin E (sIgE)-positive (titer > 0.35 U/ml) individuals among Singapore residents born in tropical and nontropical countries. (B) tIgE levels stratified by country of birth. tIgE titer for Singapore-born ethnic Chinese and Chinese migrants from China and Malaysia is shown. Median and *P*-values are indicated. (C) HDM-sIgE levels stratified by country of birth. (D) HDM-sIgE levels in migrants from China stratified by number of years' residence in Singapore. (E) Distribution of HDM-sIgE levels in migrants from Malaysia stratified by number of years in Singapore.

Discussion

This study focused on identifying the main sensitizing agents responsible for allergic airway diseases in a tropical urban environment. Allergic diseases can be caused by a number of common allergens that are present in considerable concentrations in Singapore (17). Surprisingly, we found that the response is in fact largely dominated by a single allergen, the house dust mite (HDM). A total of 70–80% of the population reacted against this allergen. The serum tIgE levels of sensitized individuals were nearly eight times higher than in nonsensitized individuals, and tIgE titers correlated directly with HDM-sIgE titers. Apparently, the environmental penetrance by the allergen is so strong that anybody who *can* mount an IgE response will react against mite allergens. On the other hand, individuals who were not sensitized by HDM usually do not develop specific IgE against any other allergen. Hence, 'HDM sensitization' is here both a true and potentially useful measure of 'atopy'.

The cause of this massive HDM response is clearly environmental. Based on our data collected from migrants, sensitization prevalence is as high in Singapore as in the neighboring tropical country of Malaysia, but much lower in the temperate climate of China: 70–80% of Singapore- or Malaysia-born Chinese react against mites, while less than 20% of the recent migrants from mainland China tested positive. Based on our previous work, Chinese individuals from Singapore and mainland China are genetically similar (18), thus excluding a major role of genetics in our observations. Instead, 'time spent in Singapore' was the strong confounding factor, with Chinese migrants who spent more than 8 years in the city-state reaching a high HDM reactivity prevalence of ~50%.

House dust mite exposure not only results in staggering specific IgE titers, it also has serious implications for disease progression. The high prevalence of HDM reactivity in Singapore- and Malaysia-born Chinese is associated with an unusually high prevalence of AR and asthma. The prevalence

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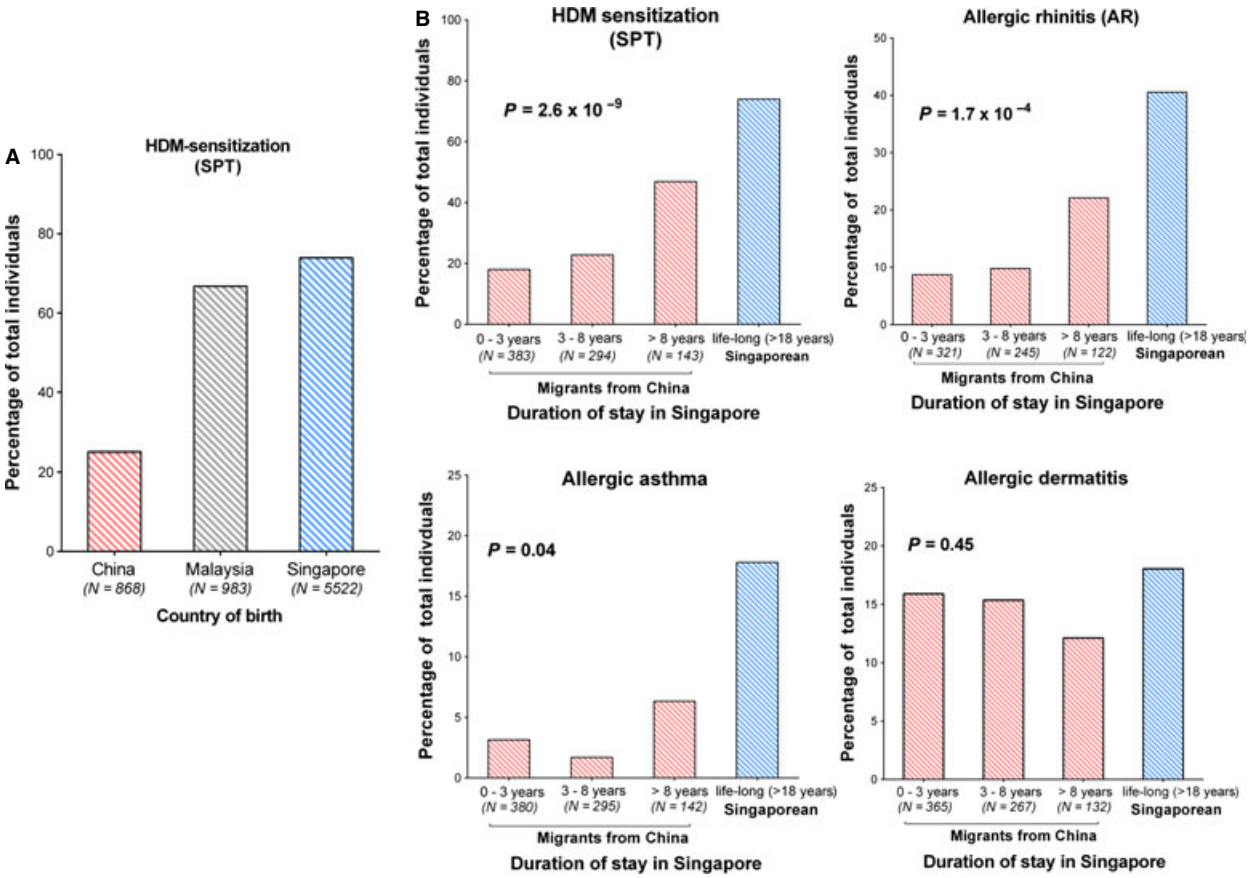

Figure 4 Influence of time of exposure to tropical urban environment on the prevalence of allergic airway diseases in the prospective validation cohort (Table 1). (A) Percentage of house dust mite (HDM)-sensitized individuals stratified by country of birth. HDM sensitization was determined by skin prick test (SPT) on ethnic Chinese born in Singapore, or on migrants from China or Malaysia. (B) HDM sensitization, allergic rhinitis (AR), asthma, and allergic dermatitis in migrants from China stratified by number of years of residence in Singapore. The percentage of individuals from China with positive SPT or allergic symptoms is shown in reference to the duration of exposure to the tropical urban environment.

of 40% and 18% for AR and asthma, respectively, rank at the top worldwide (8, 9, 19). Additionally, these symptoms were present throughout the year, with 65% of the AR individuals having persistent AR as defined by ARIA guidelines (2, 15). The causal connection between airway disease and mite-allergen exposure is implied by the increase in AR prevalence in the Chinese migrant group over time. To a weaker extent, this also applies for asthma, but not for AD; here, the rate remains unchanged, suggesting that HDM-sIgE plays only a minor role in allergic reactions of the skin.

The colloquial term for AR, 'hay fever', has its origin in the fact that pollen is the most common cause for the allergic condition in western countries. Based on our results, however, 'mite fever' would be a more accurate term in the tropical setting. Reports from countries with similar tropical climates such as Hong Kong, Malaysia, Thailand, Indonesia and Vietnam have also identified HDM as a major allergen (9, 13, 14, 20), but the cause for the shift in the trigger remains enigmatic. Pollen is present in both environments, although less seasonal in the tropics, and while *Blomia tropicalis* is indeed a species restricted to tropical regions, *Dermatophagoides pteronyssinus* is endemic worldwide. It is therefore still an open question why the allergic response in tropical Southeast Asia is so dominated by this allergen. High humidity and ambient temperatures are certainly ideal for HDM propagation (11, 12). While a consistently high HDM-load causes the perennial allergic symptomology (11, 12, 21), the same climate factors should also propagate fungi. However, based on our study, IgE-mediated allergic reactions against this outdoor allergen class are almost completely absent in Singapore, leading to the conclusion that lifestyle is an important contributor to the apparent bias toward dust mite reactivity. Clearly, in Singapore, more time is spent indoors, especially in air-conditioned spaces, which may have marked health implications. Humidity as well as rate and mode of ventilation clearly affects allergic airway phenotypes (22). Additionally, HDM-sensitized asthma patients living in homes with mechanical ventilation rather than air-conditioning exhibited overall clinical improvement through reduced HDM exposure (23).

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House dust mite allergens exert a substantial burden on direct healthcare and its related costs (24). The mono-specific trigger of the allergic airway response in tropical regions, however, offers a unique opportunity for effective prevention and treatment. In contrast to other parts of the world, where poly sensitization and seasonal variations complicate allergic disease management, interventions could focus here on a single allergen. In patients with AR, allergen-specific immunotherapy had long-term clinical benefits and also reduced the risk of developing asthma (25, 26). Allergen avoidance, in turn, is also predictably effective in managing allergic phenotypes especially in tropical environments. By simply reducing the exposure to HDM, the prevalence of both asthma and AR can be reduced here (27–29).

In conclusion, the causative agents of allergy in the tropics significantly differ from the complex etiology observed in temperate countries. Although the prevalence of allergic airway diseases is substantially higher, their mono-specific cause also offers a unique opportunity to study, treat, and manage the allergic phenotypes by specifically targeting HDM allergens.

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Author contributions

WDY, OR, and AKA performed conception and design; AKA, BL, PKJ, OR, WDY, CFT, and MP carried out analysis and interpretation; AKA, JC, AN, CFT, OR, WDY, MP, and BL were involved in drafting the manuscript for important intellectual content.

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Conflicts of interest

The authors declare that they have no conflicts of interest.

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Figure S1. Specific immunoglobulin E (IgE) levels for the house dust mite species *Dermatophagoides pteronyssinus* and *Blomia tropicalis*.

Figure S2. Influence of the time of exposure to tropical urban environment on the strength of sensitization to *Dermatophagoides pteronyssinus* and *Blomia tropicalis* in the discovery cohort (Table 1).

Figure S3. House dust mite sensitization and allergic symptoms in migrants from tropical Malaysia stratified by number of years of residence in Singapore.

Table S1. House dust mite-specific immunoglobulin E (IgE) and total serum IgE are significantly higher in individuals with allergic phenotypes.

Table S2. Association of house dust mite-specific skin prick test with allergic phenotypes.

Data S1. Definitions used in the study.

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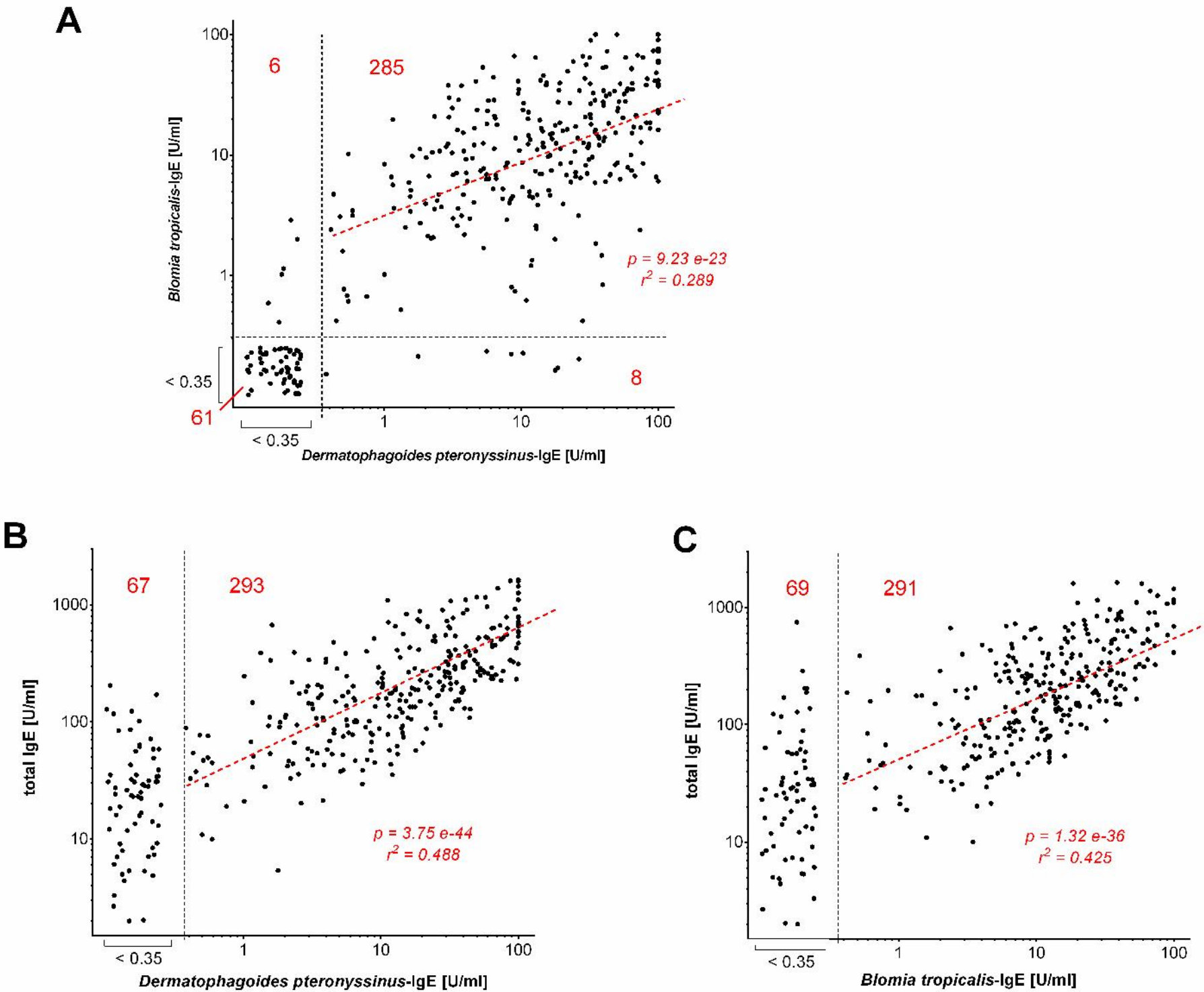
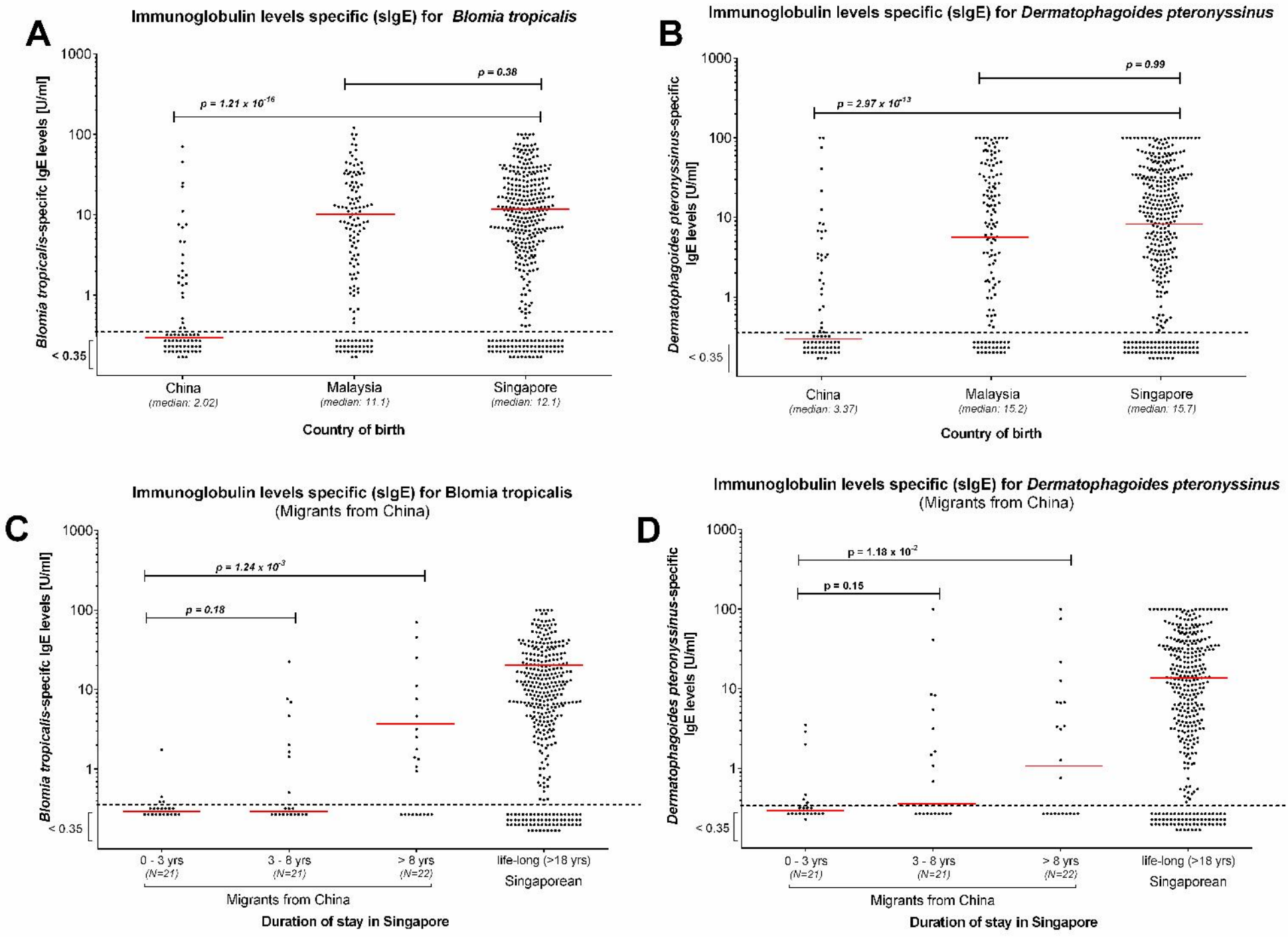
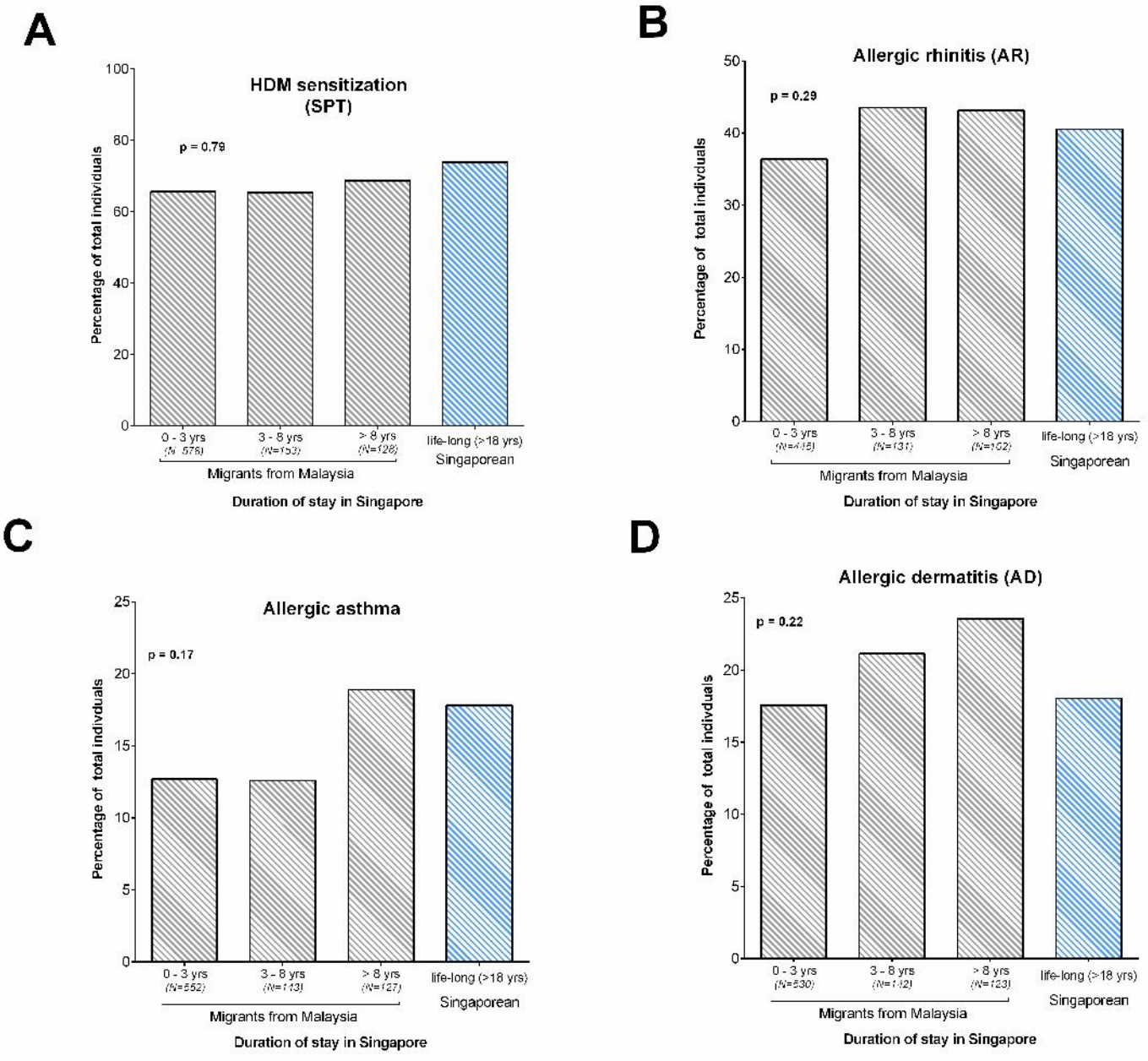

A
6
285
8
61
p = 9.23 e-23
r2 = 0.289
Blomia tropicalis-IgE [U/ml]
Dermatophagoides pteronyssinus-IgE [U/ml]
< 0.35
B
67
293
p = 3.75 e-44
r2 = 0.488
total IgE [U/ml]
Dermatophagoides pteronyssinus-IgE [U/ml]
C
69
291
p = 1.32 e-36
r2 = 0.425
total IgE [U/ml]
Blomia tropicalis-IgE [U/ml]


A
Immunoglobulin levels specific (sIgE) for Blomia tropicalis
Blomia tropicalis-specific IgE levels [U/ml]
1000
100
10
1
< 0.35
p = 1.21 x 10-16
p = 0.38
China
(median: 2.02)
Malaysia
(median: 11.1)
Singapore
(median: 12.1)
Country of birth
B
Immunoglobulin levels specific (sIgE) for Dermatophagoides pteronyssinus
Dermatophagoides pteronyssinus-specific IgE levels [U/ml]
1000
100
10
1
< 0.35
p = 2.97 x 10-13
p = 0.99
China
(median: 3.37)
Malaysia
(median: 15.2)
Singapore
(median: 15.7)
Country of birth
C
Immunoglobulin levels specific (sIgE) for Blomia tropicalis
(Migrants from China)
Blomia tropicalis-specific IgE levels [U/ml]
1000
100
10
1
< 0.35
p = 1.24 x 10-3
p = 0.18
0 - 3 yrs
(N=21)
3 - 8 yrs
(N=21)
> 8 yrs
(N=22)
life-long (>18 yrs)
Singaporean
Migrants from China
Duration of stay in Singapore
D
Immunoglobulin levels specific (sIgE) for Dermatophagoides pteronyssinus
(Migrants from China)
Dermatophagoides pteronyssinus-specific IgE levels [U/ml]
1000
100
10
1
< 0.35
p = 1.18 x 10-2
p = 0.15
0 - 3 yrs
(N=21)
3 - 8 yrs
(N=21)
> 8 yrs
(N=22)
life-long (>18 yrs)
Singaporean
Migrants from China
Duration of stay in Singapore


A
HDM sensitization (SPT)
p = 0.79
Percentage of total individuals
0 - 3 yrs
3 - 8 yrs
> 8 yrs
life-long (>18 yrs)
Singaporean
Migrants from Malaysia
Duration of stay in Singapore
B
Allergic rhinitis (AR)
p = 0.29
Percentage of total individuals
0 - 3 yrs (N=415)
3 - 8 yrs (N=131)
> 8 yrs (N=152)
life-long (>18 yrs)
Singaporean
Migrants from Malaysia
Duration of stay in Singapore
C
Allergic asthma
p = 0.17
Percentage of total individuals
0 - 3 yrs (N=552)
3 - 8 yrs (N=113)
> 8 yrs (N=127)
life-long (>18 yrs)
Singaporean
Migrants from Malaysia
Duration of stay in Singapore
D
Allergic dermatitis (AD)
p = 0.22
Percentage of total individuals
0 - 3 yrs (N=530)
3 - 8 yrs (N=132)
> 8 yrs (N=123)
life-long (>18 yrs)
Singaporean
Migrants from Malaysia
Duration of stay in Singapore

Supplementary Table 1: HDM-specific IgE and total serum IgE are significantly higher in individuals with allergic phenotypes.

	Asthma			**Allergic rhinitis**		
	Control	**Case**	**T-test P-value (diff. mean, C.I.)**	**Control**	**Case**	**T-test P-value (diff. mean, C.I.)**
No. of subjects	146	59		147	101	
Blomia tropicalis			1.471×10^{-5} (-0.45, -0.64 to -0.25)			6.564×10^{-11} (-0.59, -0.76 to -0.43)
IgE levels, mean (range)	12.44 (0.35-100)	20.79 (0.35-100)		11.68 (0.35-100)	24.06 (0.35-100)	
Dermatophagoides pteronyssinus						
IgE levels, mean (range)	20.74 (0.35-100)	32.51 (0.35-100)	2.875×10^{-6} (-0.54, -0.76 to -0.33)	14.23 (0.35-100)	36.02 (0.50-100)	6.099×10^{-18} (-0.83, -1.00 to -0.66)
Total IgE						
IgE levels, mean (range)	229.61 (2.00-1633)	339.63 (23.00-1601)	1.935×10^{-4} (-0.29, -0.45 to -0.14)	195.36 (23.00-1601)	362.88 (10.90-1601)	9.905×10^{-9} (-0.42, -0.55 to -0.28)

Stratification HDM-specific IgE or total serum IgE with allergic phenotypes. Statistics are shown for Singaporean-born Chinese (discovery cohort). P value of the T test as a well as the difference of the mean and the 95% confidence interval of the difference of the mean are listed for both asthma and allergic rhinitis. Plots are shown for the IgE titers specific for *Blomia tropicalis* and *Dermatophagoides pteronyssinus* as well as for total serum IgE.

Supplementary Table 2: Association of HDM-specific SPT with allergic phenotypes.

		Asthma			Allergic rhinitis		
	SPT status	**Control**	**Case**	**Fisher's Exact P-value (odds ratio)**	**Control**	**Case**	**Fisher's Exact P-value (odds ratio)**
Blomia tropicalis	Positive	2616 (79%)	696 (21%)	4.28×10^{-28} (3.06)	1405 (46.5%)	1614 (53.5%)	4.67×10^{-183} (11.63)
	Negative	1231 (92%)	110 (8%)		1122 (91%)	107 (9%)	
Dermatphagoides pteronyssinus	Positive	2405 (78.4%)	662 (21.6%)	1.54×10^{-28} (2.79)	1267 (45.3%)	1530 (54.7%)	8.04×10^{-165} (8.08)
	Negative	1442 (91%)	144 (9%)		1260 (87%)	191 (13%)	
Curvularia lunata	Positive	133 (75%)	45 (25%)	9.24×10^{-03} (1.57)	62 (45%)	76 (55%)	6.03×10^{-04} (1.83)
	Negative	4000 (82.5%)	845 (17.5%)		2465(60%)	1645 (40%)	
Elaeis guineensis	Positive	208 (72.5%)	79 (27.5%)	2.69×10^{-05} (1.85)	102 (42.7%)	137 (57.3%)	1.35×10^{-07} (2.01)
	Negative	3925 (83%)	811 (17%)		2425 (60%)	1584 (40%)	

Association of HDM-specific SPT with allergic phenotypes. Statistics are shown for Singaporean-born Chinese (validation cohort). P value of Fisher's exact T test as well as the odd ratios is listed for both asthma and allergic rhinitis. Correlations are shown for the SPT specific for *Blomia tropicalis* and *Dermatophagoides pteronyssinus* as well as for two control allergens *Curvularia lunata* (fungus) and *Elias guineensis* (oil palm), which were detected with lower frequency in terms of HDM positivity in Singaporeans.

Allergic airway diseases in a tropical urban environment are driven by dominant mono-specific sensitization against house dust mites

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SUPPLEMENTARY INFORMATION

Definitions used in the study

Positive sensitization as defined by skin prick test (SPT)

A wheal diameter of 3 mm or greater was considered as a positive SPT response for sensitization. A positive control with histamine and a negative control of saline were used. SPT was performed only on volunteers who had not taken any anti-allergy medication, including antihistamines, for at least 3 days prior to the test.

Positive sensitization as defined by serum screening

An allergen-specific IgE titer of >0.35 U/ml was considered indicative of positive sensitization for the allergen tested, according to the manufacturer's recommendation.

Allergic Rhinitis (AR) case

Allergic rhinitis (AR) was defined as per the ARIA (Allergic Rhinitis Impact on Asthma) guideline based on self-reported presence of two or more of these four characteristic symptoms: sneezing, runny nose, itchy nose and nose blockage, for more than an hour on most days during the past year.

Asthma case

Asthma cases were identified using the ISAAC (International Study of allergy and asthma in childohood) guidelines based on self-reported doctor-diagnosed asthma with the presence of wheezing symptomology.

Allergic dermatitis (AD) case

AD cases were identified using the U.K. Working Party's Diagnostic Criteria for Atopic Dermatitis and ISAAC guidelines based on self-reported symptoms of eczema.

Supplemental figure and table legends

Supplemental Figure 1: Specific IgE levels for the HDM species *Dermatophagoides pteronyssinus* and *Blomia tropicalis*. (A) Correlation of IgE specific for *Dermatophagoides pteronyssinus* and *Blomia tropicalis*. IgE titers in the plasma from 360 Singaporean-born ethnic Chinese were determined by ImmunoCAP ELISA. Titers <0.35 are considered non-detectable. P and r^2 values of the linear regression are indicated. (B and C) Correlation of total IgE levels with levels of IgE specific for (B) *Dermatophagoides pteronyssinus* or (C) *Blomia tropicalis*. IgE. p and r^2 values for the linear regression are indicated, numbers refer to specific IgE-positive and -negative individuals.

Supplemental Figure 2: Influence of the time of exposure to tropical urban environment on the strength of sensitization to *Dermatophagoides pteronyssinus* and *Blomia tropicalis* in the discovery cohort (Table 1). (A) *Blomia tropicalis*-specific IgE levels stratified by country of birth (Singapore, China or Malaysia). Median and p values are indicated. (B) *Dermatophagoides pteronyssinus*-specific IgE levels stratified by country of birth. (C) *Blomia tropicalis*-specific IgE levels in migrants from China stratified by number of years in Singapore. (D) *Dermatophagoides pteronyssinus*-specific IgE levels in migrants from China stratified by number of years in Singapore.

Supplemental Figure 3: HDM-sensitization and allergic symptoms in migrants from tropical Malaysia stratified by number of years of residence in Singapore. Percentage of migrants of Chinese ethnicity from Malaysia with a positive HDM-SPT (upper left panel), allergic rhinitis (upper right panel), asthma (lower left panel) or allergic dermatitis (lower right panel) is shown in reference to the response of Singaporean-born Chinese. Data were derived from the retrospective validation cohort (Table 1).

Tables

Supplemental Table 1: HDM-specific IgE and total serum IgE are significantly higher in individuals with allergic phenotypes. Statistics are shown for Singaporean-born Chinese (discovery cohort). P value of the T test, difference in the mean, and 95% confidence interval of difference in the mean are listed for both asthma and allergic rhinitis. Differences are shown for the IgE titers specific for *Blomia tropicalis* and *Dermatophagoides pteronyssinnus* as well as for total serum IgE.

Supplemental Table 2: HDM-specific SPT positivity is significantly associated with prevalence of allergic phenotypes. Statistics are shown for Singaporean-born Chinese (validation cohort). P values of Fisher's exact T test as well as the odd ratios are listed for both asthma and allergic rhinitis. Statistics are shown for the SPT specific for *Blomia tropicalis* and *Dermatophagoides pteronyssinus* as well as for two control allergens C*urvularia lunata* (fungus) and *Elais guineensis* (oil palm) with reactivity detected at lower frequency compared to the house dust mite sensitization in Singaporeans.