IgE reactivity patterns in Asian and central European cockroach-sensitized patients reveal differences in primary sensitizing allergen sources

Irene Mittermann, PhD,[a] Christian Lupinek, MD,[a] Sandra Wieser, PhD,[a] Martina Aumayr, PhD,[a] Wolfgang W. Kuchler, MD,[b] Alson W. M. Chan, MBChB, FRCPCH,[c] Tak Hong Lee, MD, ScD,[c] and Petra Zieglmayer, MD[d,e]

Vienna, Graz, and Krems, Austria; and Hong Kong, China

Background: The prevalence of cockroach (CR) sensitization and its relevance as a trigger of allergy symptoms differs greatly in different geographic areas.

Objective: This study aimed to compare molecular IgE reactivity profiles in CR-sensitized patients with perennial allergy symptoms from Hong Kong (HK) and Austria and identify the main primary sensitizers.

Methods: IgE sensitization was assessed by skin prick test and/or IgE reactivity with CR extract. Molecular IgE reactivity profiles were analyzed via multiplex assay for sensitization to allergens and extracts from CR, house dust mite (HDM), shellfish, and 3 additional insect species.

Results: HDM was the main primary sensitizer in both cohorts. In the HK group, genuine sensitization to CR was found in 45%, but none of the patients in the Austrian cohort was truly sensitized to that allergen source. Most patients from HK were cross-sensitized to other insects and/or shellfish, presumably by broad reactivity to tropomyosin and arginine kinase. About half of Austrian subjects lacked IgE to these pan-allergens, indicating co- but not cross-sensitization to insects and/or shellfish. Regarding IgE recognition frequencies, arginine kinases (64% HK, 10% Austria) and tropomyosins (42% HK, 15% Austria) were most frequently recognized; Bla g 4 (lipocalin) was detected in HK patients only (42%). Tropomyosin (Per a 7) was significantly more frequently recognized in patients with asthma. Sera from HDM-sensitized subjects from HK showed a higher proportion of sensitization to minor mite allergens.

Conclusion: Molecular profiling identified differences between CR-sensitized allergic patients from HK and Austria in terms of primary sensitizers and molecular IgE reactivity patterns. Tropomyosin from American cockroach (Per a 7) was shown to be significantly associated with asthma symptoms and might be suitable as biomarker for more severe respiratory allergy symptoms. (J Allergy Clin Immunol Global 2022;1:145-53.)

Key words: *Component-resolved diagnosis, cockroach sensitization, multiplex testing, microarray, arginine kinase, tropomyosin, cross-reactivity, edible insect sensitization, primary sensitizer*

Many people spend more than 90% of their lives in indoor environments, so it is unsurprising that hypersensitivity reactions to indoor allergens such as house dust mites (HDM), domestic animal dander, cockroach (CR), and mold spores are on the rise in inner-city areas. In genetically predisposed individuals, inhalation of CR allergens was established as an important cause of asthma 50 years ago[1] and has been confirmed in more recent studies.[2-4] Whereas in southern China considerable amounts of CR allergens were detected in households,[5,6] reflected by their paramount role as sensitizer to indoor allergens,[7-10] CR plays only a minor role as an allergen source in Europe.[11-16] After inhaling CR extract, CR-sensitized asthmatic patients experience early- and late-phase allergic reactions.[1] In the United States, 60% to 80% of inner-city children with asthma are sensitized to CR allergens, which constitutes a risk factor for hospitalization, a higher number of medical visits, and more reported symptoms.[17-19] Remarkably, in those regions, exposure to CR allergens appears to have a greater impact on asthma morbidity than allergy to HDM or furry animals.[17,20] It has been demonstrated that there is a significant association between exposure to CR allergens in the first 3 months of life and the development of repeated wheeze among children in different US cities.[21]

The most common domestic CR species associated with allergy are *Blattella germanica* (German cockroach) and *Periplaneta americana* (American cockroach). Over the last 25 years, several important CR allergens have been purified and characterized; they can now be used for the systematic assessment of the sensitization profiles of allergic individuals.[19,22-28] Component-resolved diagnosis allows for distinguishing between genuine sensitizations, cosensitizations, and cross-reactivities for different allergen sources.[29] With this approach, it has been shown that CR-allergic patients present variable sensitization profiles, without defined immunodominant major CR allergens.[19,30-32] Thus, the importance of individual CR allergens in causing sensitization varies in different areas of the world, influenced by climatic, other

From [a]MacroArray Diagnostics, Vienna, [b]ENT practice, Graz, [c]the Allergy Centre, Hong Kong Sanatorium and Hospital, Hong Kong, [d]the Vienna Challenge Chamber, Vienna, and [e]Karl Landsteiner University, Krems.

The first 2 authors contributed equally to this article, and both should be considered first author.

Disclosure of potential conflict of interest: I. Mittermann, C. Lupinek, S. Wieser, and M. Aumayr are employees of MacroArray Diagnostics, Vienna, Austria; and P. Zieglmayer received honoraria from MacroArray Diagnostics, Vienna, Austria. The rest of the authors declare that they have no relevant conflicts of interest.

Received for publication January 11, 2022; revised March 17, 2022; accepted for publication April 8, 2022.

Available online April 22, 2022.

Corresponding author: Petra Zieglmayer, MD, Vienna Challenge Chamber, Huetteldorferstrasse 44, 1150 Vienna, Austria. E-mail: petra@zieglmayer.at.

The CrossMark symbol notifies online readers when updates have been made to the article such as errata or minor corrections

2772-8293

https://doi.org/10.1016/j.jacig.2022.04.003

Abbreviations used
AD: Atopic dermatitis
AIT: Allergen immunotherapy
CCD: Cross-reactive carbohydrate determinant
CR: Cockroach
HDM: House dust mite
HK: Hong Kong
kU/L: Kilounits per liter
kUA/L: Kilounits of allergen-specific IgE per liter

environmental, and dietary factors, as well as, presumably, by genetic predisposition. Moreover, strong IgE cross-reactivity between CR, HDM, and shellfish allergens has been described, in manycases mediated by pan-allergens, such as tropomyosins or arginine kinases.[25,27,33]

The aim of the present study was to assess potential differences in IgE sensitization profiles of CR-sensitized patients with perennial respiratory allergy symptoms from 2 different geographic areas: coastal China (Hong Kong, HK) and central Europe (Austria). We used a comprehensive panel of CR allergens and assessed patients' IgE reactivity patterns by using the ELISA-based multiplex allergy test ALEX^2, where only small volumes of serum samples are needed to determine sensitization profiles to many seasonal and perennial allergens in a single analysis.[34] On the basis of these comprehensive IgE reactivity patterns, we determined the primary sensitizing allergen sources and differentiated between co- and cross-sensitizations in the studied cohorts.

METHODS

Study population

For the present study, patients with the diagnosis of CR allergy were enrolled. Their diagnosis was based, according to ARIA[35] and GINA[36] guidelines, on the presence of perennial rhinitis and/or asthma and confirmed IgE sensitization to CR. Sensitization to CR was assessed by measurement of IgE reactivity (ImmunoCAP i6, Thermo Scientific, Uppsala, Sweden) and/or by skin prick test to CR extract, in line with current guidelines.[37] In addition, IgE reactivity to cross-reactive carbohydrate determinants (CCDs) was quantified in the Austrian cohort by ImmunoCAP measurements (MUXF3, ImmunoCAP o214). Patients from Austria were recruited from an outpatient service in Styria (eastern Austria), and patients from HK were selected at the Allergy Centre of the Hong Kong Sanatorium and Hospital (China). The study was approved by the respective local ethics committees.

Microarray-based IgE antibody measurements

Total IgE and levels of specific IgE to a comprehensive panel of CR, mite, and shellfish allergens, as well as to extracts from house cricket (*Acheta domesticus*), migratory locust (*Locusta migratoria*), and mealworm (*Tenebrio molitor*), were measured via ALEX^2 multiplex testing (MacroArray Diagnostics, Vienna, Austria). In this serologic test system, purified allergens and extracts are first coupled to polystyrene nanobeads, which then are spotted onto nitrocellulose membrane. For IgE measurement, ALEX^2 chips were incubated with 0.5 mL of 1:5 diluted serum under agitation. Notably, the serum diluent contains a CCD inhibitor. After incubation for 2 hours, the chips were washed, and an anti-human, alkaline phosphatase–labeled IgE antibody was added for 30 minutes. After another cycle of washing, the enzyme substrate was added, and, after drying the membranes, the intensity of the color reaction for each allergen spot was measured by a charge coupled device camera. Results were analyzed by RAPTOR Analysis Software (MacroArray Diagnostics), with total IgE levels expressed as kilounits per liter (kU/L) and allergen-specific IgE levels as kilounits of allergen-specific IgE per liter (kU_A/L). Values ≥0.3 kU_A/L were considered positive.[34,38]

Statistical analysis

To compare frequencies of IgE reactivities to spotted allergen molecules in the Austrian and HK study populations, 2-sided asymptotic P values were calculated by chi-square test. $P \leq .05$ was considered statistically significant. Correlation between levels of serum specific IgE to allergen extracts or individual allergens was analyzed according to Pearson. Differences in IgE levels among the sera from HK and Austria were analyzed by the Mann-Whitney U test. For statistical analyses, calculators from Social Science Statistics (www.socscistatistics.com, 2018) were used.

RESULTS

Demographic and clinical characterization of study populations from HK and Austria

In total, 33 individuals from HK and 62 subjects from Austria were enrolled with a diagnosis of allergy to CR that was based on IgE sensitization to CR extract, perennial allergic rhinitis, and/or allergic asthma (Table I). All individuals comprising the Austrian study group were IgE positive to CR extract (*Blattella germanica,* ImmunoCAP i6, Thermo Scientific), with a median value of 0.995 kU_A/L, ranging from 0.35 to 11.1 kU_A/L ($Q_{25\%}$ = 0.645 kU_A/L, $Q_{75\%}$ = 1.725 kU_A/L). Patients from HK were randomly chosen from a cohort of patients of the allergy unit of the Hong Kong Sanatorium and Hospital. For the latter, IgE sensitization to CR was determined by measurement of CR-specific serum IgE or by positive skin prick test to CR extract.

In the HK cohort, 4 of 33 patients were subjected to allergen immunotherapy (AIT; 3 subjects to HDM, 1 subject to HDM and cat) at the time of serum collection; in the Austrian cohort, 8 subjects had completed AIT before enrollment and another 11 were undergoing treatment when they were enrolled (14 to HDM, 4 to CR and 1 to both).

There were no significant differences between the 2 study groups regarding sex distribution (Table I). However, median age as well as total serum IgE levels in the Austrian (230 kU/L) and HK (2097 kU/L) groups were significantly different, with the latter reflecting a higher percentage of atopic dermatitis (AD) patients in the HK group (85%, vs 3 recorded cases of AD in the Austrian cohort). In terms of respiratory symptoms, the 2 groups were comparable (Table I).

Multiplex IgE detection by ALEX^2 revealed distinct IgE reactivity profiles to CR and HDM in study populations from HK and Austria

IgE sensitization to at least 1 of the 6 CR allergens (Bla g 1, 2, 4, 5, and 9; Per a 7) and/or to CR extract from *Periplaneta americana* was found in all 33 patients from HK. In contrast, in the Austrian study population, at least 1 IgE component or extract from CR was detectable in only 20 (32%) of 62 tested sera (Table II).

In both cohorts, the highest rates of IgE sensitization were found for pan-allergens—that is, arginine kinase (Bla g 9) and tropomyosin (Per a 7; Table II, Fig 1). From the CR-specific allergens, Bla g 4 (calycin, lipocalin) was positive in 42%, and Bla g 1, 2, and 5 occurred at much lower rates for HK subjects but for none of the subjects of the Austrian cohort.

By analyzing the sensitization profile to 14 HDM and storage mite allergens (Der p 1, 2, 5, 7, 10, 11, 20, 21, and 23; Der f 1, 2;

TABLE I. Demographic and clinical characterization of study cohorts from HK and Austria

Characteristic	HK (n = 33)	Austria (n = 62)
Sex, F/M (%F)	12/21 (36)	26/36 (42)
Age (years), median (range)*	24 (6-39)	38.5 (11-76)
Patients with:		
Rhinitis only	23 (70)	31 (50)
Both rhinitis and asthma	7 (21)	19 (31)
Asthma only	3 (9)	12 (19)
AD*	28 (85)	3 (5)
Total serum IgE (kU/L), median (range)*	2097 (78-3048)	230 (7-1872)

Data are presented as nos. (%) unless otherwise indicated.
*Statistically significant differences between cohorts ($P < .001$).

Blo t 5, 10, and 21), 97% of the sera from the HK cohort and 71% from the Austrian cohort showed IgE antibodies to at least 1 mite allergen (Table II). Allergens from the species *Dermatophagoides pteronyssinus* (97% HK, 69% Austria) and *Dermatophagoides farinae* (97% HK, 55% Austria) were recognized by most patients, followed by *Blomia tropicalis* (82% HK, 21% Austria; Table II). In patients from HK, Der p 1 or Der f 1 alone was able to diagnose 32 of 33 individuals as being HDM sensitized; only 1 patient showed a negative result with all mite allergens tested. In the Austrian group, a combination of Der p 1, Der p 2, and Der p 23 identified 58% as sensitized to HDM. Remarkably, the pan-allergens Der p 10 and Der p 20, as well as minor allergens (Der p 5, 7, and 21), were IgE positive in around 50% or even more of the sera from HK, whereas almost no IgE sensitization was found to Der p 11. In contrast, IgE reactivity to pan- and minor allergens from HDM was detected at much lower frequencies in the Austrian cohort (Fig 2, Table II).

Group 2 mite allergens showed a high rate of IgE reactivity

In both cohorts, the prevalence of IgE sensitization to group 2 allergens from storage mites was high and was dominated by Lep d 2, followed by less frequent sensitization to Gly d 2 and Tyr p 2 (Table II, and see Table E1 in this article's Online Repository at www.jaci-global.org). Notably, 5% of patients from Austria and 9% from HK showed exclusive species-specific IgE reactivity to Lep d 2. In both cohorts, the majority of patients were cross-sensitized to Der p 2, Der f 2, and Lep d 2; most of the patients from HK were additionally sensitized to Gly d 2. IgE levels to storage mite allergens Lep d 2, Gly d 2, or Tyr p 2 were significantly lower than IgE levels to group 2 HDM allergens Der p 2 or Der f 2 in both cohorts (Table E1).

CR is not a relevant primary sensitizing allergen source in Austria

The ALEX^2 array comprises marker allergens for genuine sensitization to HDM (Der p 1, 2, 23) and CR (Bla g 1, 2).[39] Because the role of Bla g 4 and Bla g 5 remains less clear, we considered these 2 allergens separately in our analysis (see Table E2 in the Online Repository at www.jaci-global.org). Edible insects (house cricket, migratory locust, mealworm) were represented by extracts, and shellfish by extracts (oyster, lobster, mussel, shrimp) and pan-allergens (Pen m 1-4; Ani s 3), respectively. This allows for identification of genuine sensitization to HDM and CR, while for shellfish and edible insects (hereafter just termed insects), cosensitization can only be deduced from lack of IgE reactivity to other, potentially cross-reactive allergen sources.

Fig 3, *A,* illustrates the overlap, and Table E2 shows the different patterns of sensitization to the highly cross-reactive allergen sources HDM, CR, shellfish, and insects. In the Austrian cohort, although enrollment was based on IgE reactivity to CR extract, no sensitization to marker allergens from CR was detected, indicating that reactivity to the extract was due to cross-sensitization (Table E2, *A*). In contrast, most patients (n = 36, 58%) showed genuine IgE reactivity to at least 1 specific marker allergen from HDM. Nine of these subjects were also positive to pan-allergens, which probably accounted for cross-reactivity with extracts from CR (n = 3), insects (n = 7), or shellfish (n = 8). However, 27 patients with genuine HDM sensitization notably were IgE negative to pan-allergens; therefore, sensitization to extracts from insects (n = 7) or shellfish (n = 10) could represent primary IgE sensitization to those allergen sources (Table E2, *A*). One patient showed IgE reactivity to extracts from the mite species *Acarus siro* and *Tyrophagus putrescentiae* but was negative to HDM markers, as well as to tropomyosin and arginine kinase (Table E2, *A*). This patient was also positive to extracts from insects and CR.

To identify other possible primary sensitizing allergen sources apart from HDM, subjects with IgE to at least 1 HDM marker allergen were omitted (Fig 3, *B, left,* Table E2, *A*). Among the remaining 26 patients (Table E2, *A*), 7 exhibited broad IgE reactivity to pan-allergens from HDM, CR, and shellfish, with 6 patients being positive to extracts from CR, shellfish, and/or insects and 1 who was positive to pan-allergens only. In these cases, it was impossible to define the possible primary sensitizing allergen sources. When omitting the pan-allergens tropomyosin and arginine kinase, 6 were exclusively positive to extracts from insects and 2 to shellfish extracts (Fig 3, *C,* Table E2, *A*) but negative to pan-allergens, possibly indicating primary sensitization to the respective allergen sources. The remainder (n = 6) exhibited co- or cross-reactivity between insects, shellfish, and/or CR.

Of the 4 patients without serum IgE to HDM, CR, insects, or seafood, 2 were positive to allergens from various pollens and furry animals, and 2 were negative to any allergen in the ALEX^2 array. Notably, 25% of Austrian sera contained CCD-specific IgE as determined by ImmunoCAP.

Almost all patients from HK were sensitized to HDM with frequent cosensitization to CR

In our HK cohort, each study participant (n = 33) had IgE to CR markers, pan-allergens, and/or extracts (Fig 3, *right,* Table E2, *B*). Five patients were IgE sensitized to CR markers (Bla g 1, 2), and another 10 subjects were sensitized to 2 additional putative CR markers (Bla g 4, 5), indicating 15 cases (45%) of genuine sensitization to CR. However, 32 subjects (97%) had IgE to markers for HDM sensitization, with 15 patients being co-sensitized to CR. One case of genuine IgE sensitization to HDM showed concomitant reactivity to extracts from insects and CR but negativity to CR markers and pan-allergens (Table E2, *B*). This pattern could reflect cosensitization to HDM and insects or cross-sensitization via other, less prevalent pan-allergens.

TABLE II. Frequencies of IgE sensitization to allergens or extracts from CR, mites, shellfish, or edible insects in patients from HK and Austria

Allergen or allergen extract	HK (n = 33)	Austria (n = 62)	*P* values	Species, common name, allergen family, and/or function
Cockroach				
Bla g 1	2 (6)	0	.049*	*Blattella germanica,* German cockroach, group 1
Bla g 2	3 (9)	0	.016*	Aspartic protease
Bla g 4	14 (42)	0	≤.001*	Calycin, lipocalin
Bla g 5	1 (3)	0	.171	Glutathione S-transferase
Bla g 9	21 (64)	6 (10)	≤.001*	Arginine kinase
Any Bla g	27 (82)	6 (10)	≤.001*	
Per a 7	14 (42)	9 (15)	.003*	*Periplaneta americana,* American cockroach, tropomyosin
Per a (extract)	9 (27)	12 (19)	.378	
Any Bla g, Per a 7, and/or Per a extract	33 (100)	20 (32)	≤.001*	
Mite				
Der p 1	32 (97)	22 (35)	≤.001*	*Dermatophagoides pteronyssinus,* European HDM, group 1, cysteine protease
Der p 2	29 (88)	30 (48)	≤.001*	Group 2, NPC2
Der p 5	15 (45)	7 (11)	≤.001*	Group 5/21
Der p 7	17 (52)	10 (16)	≤.001*	Group 7
Der p 10	17 (52)	9 (15)	≤.001*	Group 10, tropomyosin
Der p 11	1 (3)	0	.171	Group 11, paramyosin
Der p 20	20 (61)	6 (10)	≤.001*	Group 20, arginine kinase
Der p 21	20 (61)	11 (18)	≤.001*	Group 5/21
Der p 23	26 (79)	19 (31)	≤.001*	Group 23, peritrophin-like protein
Any Der p	32 (97)	43 (69)	.002*	
Der f 1	32 (97)	22 (35)	≤.001*	*Dermatophagoides farinae,* American HDM, group 1, cysteine protease
Der f 2	29 (88)	30 (48)	≤.001*	Group 2, NPC2
Any Der f	32 (97)	34 (55)	≤.001*	
Blo t 5	21 (64)	7 (11)	≤.001*	*Blomia tropicalis,* storage mite, group 5/21
Blo t 10	17 (52)	10 (16)	≤.001*	Group 10, tropomyosin
Blo t 21	16 (48)	2 (3)	≤.001*	Group 5/21
Any Blo t	27 (82)	13 (21)	≤.001*	
Any Der p, Der f, and/or Blo t	32 (97)	44 (71)	.04*	
Tyr p 2	12 (36)	2 (3)	≤.001*	*Tyrophagus putrescentiae,* storage mite, group 2, NPC2
Lep d 2	31 (94)	31 (50)	≤.001*	*Lepidoglyphus destructor,* storage mite, group 2, NPC2
Gly d 2	26 (79)	15 (24)	≤.001*	*Glycyphagus domesticus,* storage mite, group 2, NPC2
Any Gly d, Lep d and/or Tyr p	32 (97)	31 (50)	≤.001*	
Shellfish				
Pen m 1	13 (39)	9 (15)	.006*	*Penaeus monodon,* black tiger shrimp, tropomyosin
Pen m 2	16 (48)	5 (8)	≤.001*	Arginine kinase
Pen m 3	2 (6)	0	.049*	Myosin light chain
Pen m 4	3 (9)	2 (3)	.207	Sarcoplasmic calcium-binding protein
Any Pen m	23 (70)	13 (21)	≤.001*	
Ani s 3	15 (45)	9 (15)	≤.001*	*Anisakis simplex,* herring worm, tropomyosin
Ost e (extract)	11 (33)	22 (35)	.835	*Ostrea edulis,* oyster
Hom g (extract)	17 (52)	24 (39)	.229	*Homarus gammarus,* lobster
Pan b (extract)	10 (30)	18 (29)	.898	*Pandalus borealis,* northern prawn
Myt e (extract)	8 (24)	5 (8)	.03*	*Mytilus edulis,* common mussel
Insects				
Ach d (extract)	23 (70)	31 (50)	.067	*Acheta domesticus,* house cricket
Loc m (extract)	21 (64)	26 (42)	.043*	*Locusta migratoria,* migratory locust
Ten m (extract)	19 (58)	28 (45)	.248	*Tenebrio molitor,* mealworm

Data are presented as nos. (%).
*Statistically significant differences between cohorts.

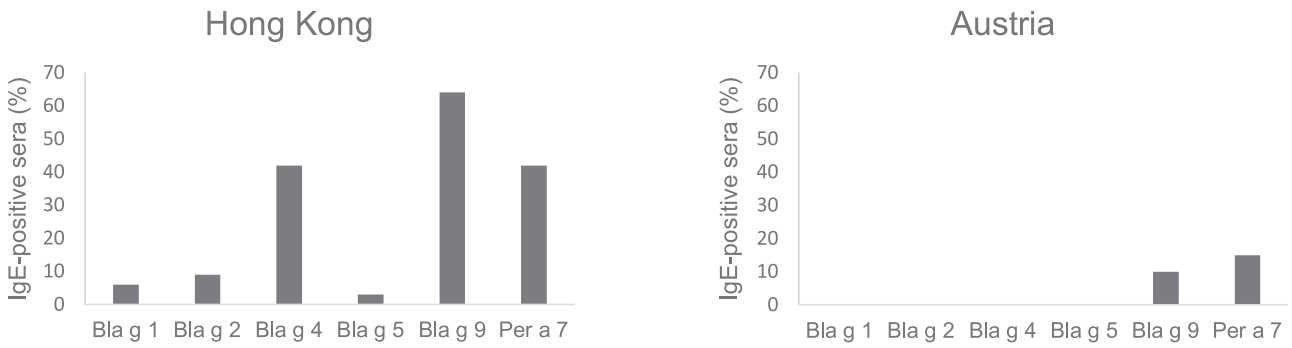

FIG 1. IgE recognition profiles to CR allergens. Percentages of IgE binding (*y-axes*) to individual CR allergens (*x-axes*) in patients from HK (*left*) and Austria (*right*).

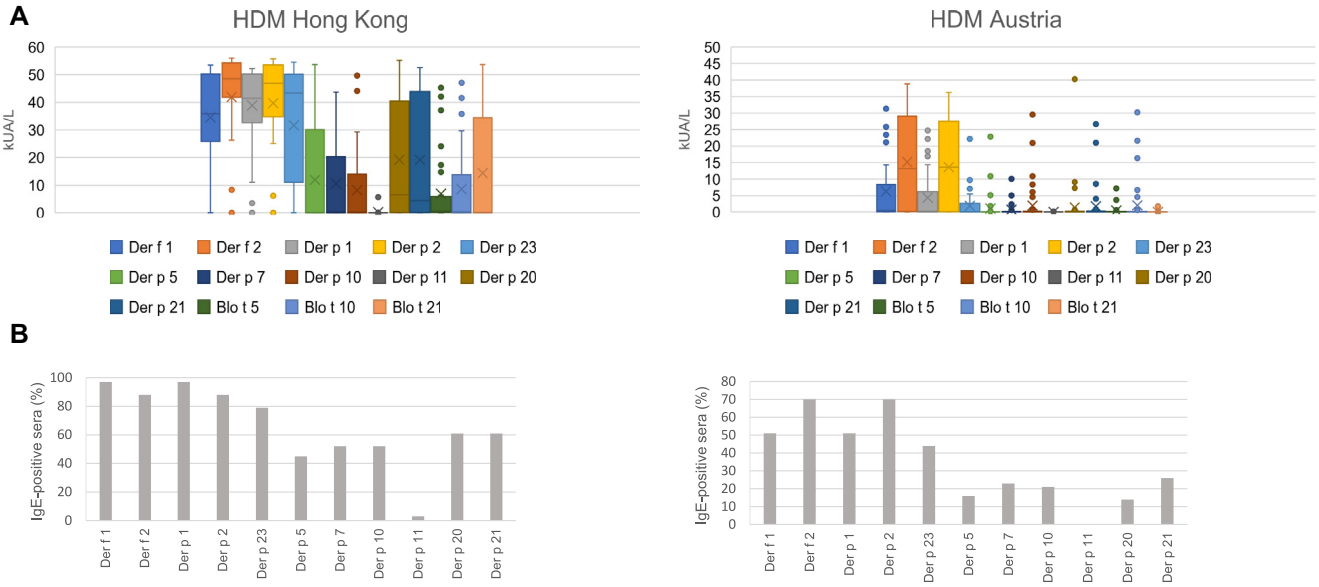

FIG 2. IgE recognition profiles to HDM allergens in HDM-positive patients from HK and Austria. (**A**) IgE levels (*y-axes,* kU_A/L) and (**B**) IgE binding prevalence (*y-axes,* percentage) to individual HDM allergens are displayed for mite-sensitized patients of the HK (*left*) and Austrian study population (*right*). *Box plots* show medians, and first and third quartiles; *whiskers,* minima and maxima. In addition, means (*x* within the respective boxes) are displayed; outliers are represented by *circles.*

However, patients with genuine sensitization to HDM and/or CR (n = 32) were positive to pan-allergens in all but 3 cases. In these 29 patients, it was impossible to ascertain whether concomitant reactivity to extracts from insects (n = 23) or shellfish (n = 20) reflected co- or cross-sensitization to these allergen sources.

Allergen-specific IgE levels to tropomyosins, arginine kinases, and different insect extracts were significantly correlated

Both tropomyosin (Per a 7) and arginine kinase from CR (Bla g 9) showed significantly higher IgE reactivities in the cohort from HK compared to Austria (Table II). IgE levels to Per a 7 were significantly correlated with IgE levels to tropomyosins from mite (Der p 10; Blo t 10), shrimp (Pen m 1), and herring worm (Ani s 3; Fig 4, *A*). The same was observed for IgE levels to Bla g 9 and Der p 20 or Pen m 2, respectively (Fig 4, *B*).

Likewise, IgE levels to extracts from house cricket (*Acheta domesticus*) showed high correlation with values obtained with extracts from grasshopper (*Locusta migratoria*) and mealworm (*Tenebrio molitor,* Fig 4, *C*).

IgE sensitization to Per a 7 was significantly higher in patients with asthma than patients without asthma

A significant difference in IgE sensitization frequencies to Per a 7 was found between CR-allergic patients from HK with and without asthma. Seventy percent of asthma patients showed Per a 7–specific IgE, compared to 26% in the group without asthma (Fig 5, *A*). In patients with asthma, rates of IgE positivity were also higher for tropomyosins from HDM, shrimp, and herring worm, but these differences were not statistically significant (Fig 5, *A*).

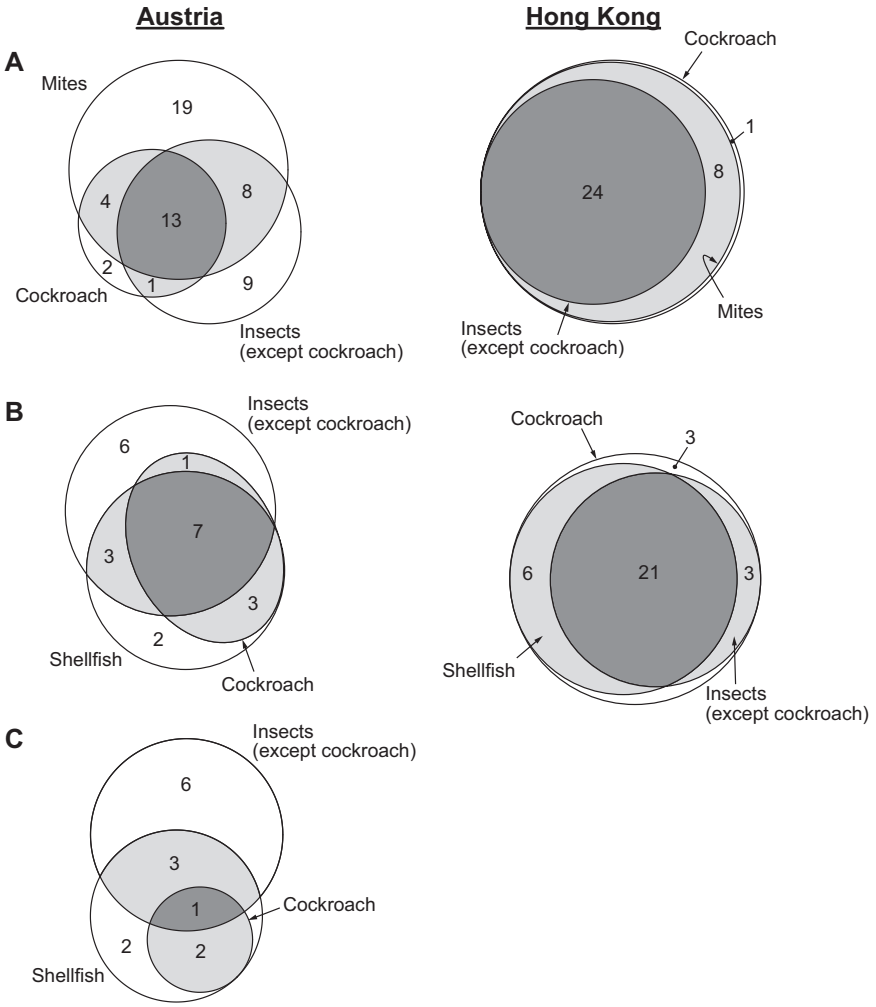

FIG 3. Euler diagrams showing overlap of IgE sensitization to (**A**) CR, insects other than CR, and mites for study participants from Austria (*left*) and HK (*right*). IgE reactivity to marker or pan-allergens, as well as to extracts from the respective allergen source, were considered. (**B**) IgE reactivity to CR, insects other than CR, and shellfish. For the Austrian cohort, only patients negative to mite-specific marker allergens (Der p 1, 2, and/or 23) were included (*B*). (**C**) Overlap of IgE reactivity to CR, insects other than CR, and shellfish for Austrian patients negative to HDM marker allergens and to the pan-allergens tropomyosin and arginine kinase.

For arginine kinases, no statistically significant differences were found between those with and without asthma (Fig 5, *B*).

DISCUSSION

To our knowledge, our study is the first to analyze IgE sensitization profiles to a panel of CR allergens in sera from patients with allergic rhinitis and/or asthma from 2 distinct geographic regions with different climatic conditions and allergenic environments: coastal Asia (HK) and central Europe (Austria). While CR had been identified as one of the most relevant indoor allergen sources in southern China,[7-10] it is considered of minor importance in central Europe. In the present study, we used a multiplex allergy test to determine IgE reactivity to allergen molecules and extracts from CR and other indoor allergens, as well as from food allergen sources.

To date, no unequivocal marker allergens for sensitization to CR have been identified, and IgE reactivity patterns of CR allergic subjects vary greatly.[19,40] However, IgE to Bla g 1 and 2, and possibly also Bla g 4 and 5, is considered specific for CR, especially for the differential diagnosis of co- or cross-sensitization to HDM, while tropomyosins and arginine kinases are broadly cross-reactive pan-allergens.

Our results showed that the common environmental exposure to allergens from CR and HDM[5,6] was clearly depicted in the HK study population by high rates of genuine sensitization, amounting to 45% and 97%, respectively. As we expected, we also observed frequent cross-sensitization to shellfish allergens[33] and extracts from 3 other edible insect species, which is attributable to a high percentage of IgE reactivity to pan-allergens such as tropomyosins or arginine kinases (Fig 3, Table II, Table E2, *B*).

In the Austrian study cohort, HDM was also identified as a main primary sensitizer (58%), but none of the patients were genuinely sensitized to CR (Table E2, *A*). In contrast to 100% IgE sensitization to CR extract determined during enrollment, only 22 subjects (35%) exhibited reactivity to CR pan-allergens or extract, or pan-allergens from HDM or shellfish on the ALEX^2 test.[39] However, serum IgE levels to CR measured by ImmunoCAP during

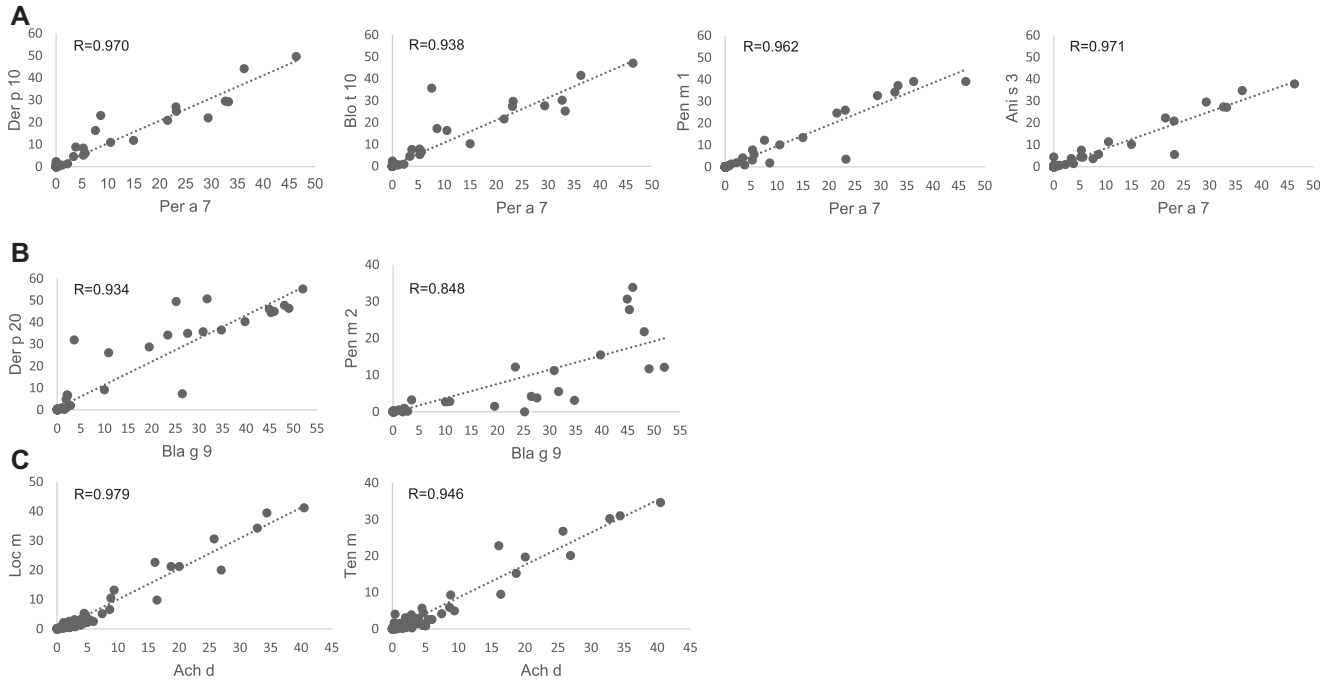

FIG 4. Correlations of serum IgE levels (*x- and y-axes,* kU_A/L) to (**A**) tropomyosin from American CR (Per a 7), mite (Der p 10, Blo t 10), shrimp (Pen m 1), and herring worm (Ani s 3), (**B**) Arginine kinase Bla g 9, Der p 20, and Pen m 2 from the same respective species, and (**C**) extracts from *Acheta domesticus, Locusta migratoria,* and *Tenebrio molitor*. *Scatterplots* show values from individual patients; *dotted lines* illustrate correlation between IgE levels to the respective allergen molecules or extracts, respectively. Pearson correlation coefficients are indicated for each chart ($P < .001$ for each pairwise analysis).

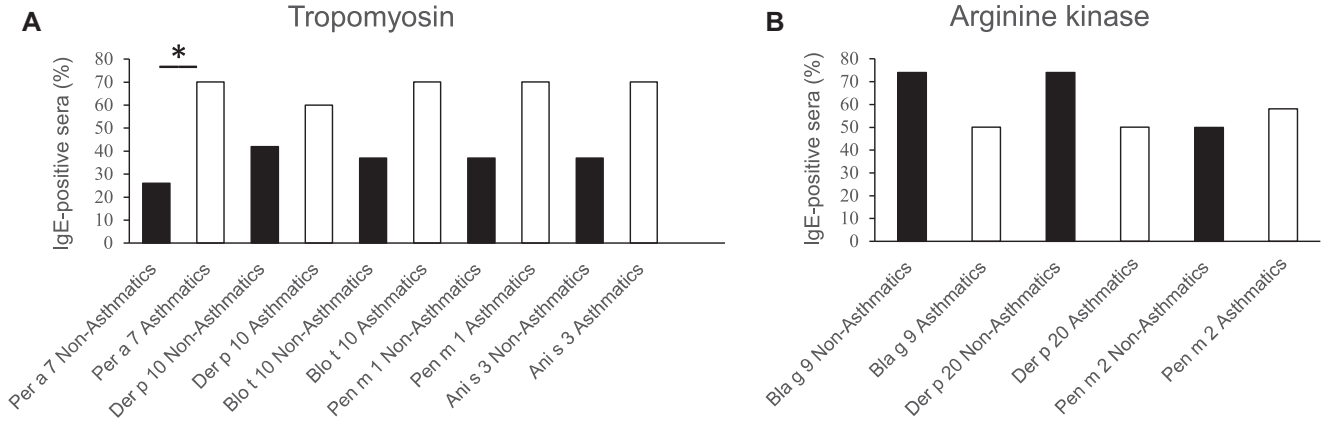

FIG 5. IgE recognition of tropomyosin and arginine kinase from different sources among patients with and without asthma. IgE binding prevalence (*y-axes,* percentage) to (**A**) tropomyosin from CR (Per a 7), mite (Der p 10, Blo t 10), shrimp (Pen m 1), and herring worm (Ani s 3) and (**B**) arginine kinase from CR (Bla g 9), HDM (Der p 20), and shrimp (Pen m 2) for nonasthmatic (*black bars*) and asthmatic (*white bars*) CR-allergic patients from HK. *Statistically significant differences between the 2 groups ($P \leq .05$).

recruitment had been relatively low in Austrian patients. Therefore, it is conceivable that a considerable part of such low results was caused by nonspecific IgE reactivity with the CR extract[41] or the cellulose matrix of the CAP system,[42] which is supported by 25% of the Austrian samples containing CCD-specific IgE. In the ALEX^2 test system, a CCD blocker is comprised in the standard protocol, preventing such false-positive results. Another possible cause of this discrepancy could be IgE sensitization to other pan-allergens, such as troponin C (Bla g 6) or myosin light chain (Bla g 8).

In total, a high percentage of mite-sensitized allergic subjects showed concomitant IgE reactivity to CR, shellfish, or insects (39%, 59%, 48%) in the Austrian cohort, in line with previous data from Iceland (33% for CR, 58% for shellfish).[43] Insects and shellfish were identified as possible primary sensitizing allergen sources in subjects without IgE reactivity to pan-allergens and

CR extract, and positive to either extracts from insects (n = 9, 3 cosensitized to HDM markers) or shellfish (n = 8, 6 cosensitized to HDM markers; Table E2, *A*). Another 6 subjects negative to pan-allergens were positive to both insects and shellfish, which does not allow for identification of the primary sensitizer. The observed surprisingly high rates of putative primary sensitization to insects and/or shellfish in the Austrian cohort could be partly explained by IgE reactivity to other, less prevalent pan-allergens. However, in cases without IgE reactivity to the most prevalent indoor allergen sources despite unequivocal clinical history, primary sensitization to (edible) insects or shellfish should be considered in allergy diagnostics. While insects are part of the daily diet in many Asian countries, they have become available at the European market only recently.[44] It is therefore more likely that Austrian patients were sensitized by respiratory exposure to insect allergens.

The observed discrepancy between HK and Austria regarding the role of CR as a primary sensitizer is in agreement with the overall lower relevance of CR as an allergen source in Europe.[11-14] In addition to regional differences in allergen exposure, genetic differences between the studied populations might add to the observed results regarding rates of IgE sensitization to CR.[2-4]

Tropomyosin[45-48] and arginine kinase[27,32,49-51] have been previously identified as cross-reactive pan-allergens in invertebrates that are associated with allergy to various shellfish.[52-56] Detailed analysis of IgE reactivities to tropomyosins[43-46] and arginine kinases showed more than 50% IgE positivity for both allergens in the HK cohort (Table II), with a strong correlation between IgE levels to the respective allergens from different species (Fig 4). The high prevalence of positivity to these pan-allergens in the HK cohort might be due to the frequent consumption of shellfish in this region, but also to common respiratory exposure to tropomyosins and arginine kinases from HDM.[48] Notably, we observed a significant association of sensitization to Per a 7, but not to arginine kinases, with more severe respiratory symptoms, such as allergic asthma (Fig 5). However, as a result of the small number of patients with asthma (n = 10), further studies are needed to evaluate the relevance of those pan-allergens as possible biomarkers for asthma.

Study limitations

Our 2 study populations differed considerably in percentages of AD patients. However, even though AD conveys a higher general propensity for IgE sensitization, the latter always depends on exposure, and thus on the prevalence of the respective allergen source in the area under investigation.[57] In addition, a study in southern China showed that rates of sensitization to CR, HDM, and shellfish are similar in patients with respiratory allergy symptoms compared to AD patients.[7] In line with our findings, it was previously reported that rates of concomitant sensitization to CR, HDM, and shellfish are high in Chinese allergic populations,[7-9] including exclusively respiratory allergies.[10] Therefore, we consider differences in the sensitization rates of our 2 study groups to reflect the particular regional characteristics of local allergen exposure.

Another possible limitation could be differences in rates of immunotherapy between the 2 cohorts (HK 12%, Austria 31%). However, none of the patients from HK and only 5 subjects from Austria received AIT to CR. Even despite receipt of AIT to CR, none of those 5 subjects has mounted an IgE response to marker allergens from CR, which indicates that sensitization rates to CR were not affected by AIT.

Conclusion

A high prevalence of genuine sensitization to HDM was observed in both study groups, whereas true sensitization to CR was observed in a considerable proportion of patients from HK, but not in any Austrian study participant. In Austrian subjects, insects and shellfish were identified as the possible primary sensitizing allergen source in an unexpectedly high number of cases.

We thank Peter Forstenlechner (MacroArray Diagnostics, Vienna, Austria) and Hammer Tsui (T-Medi, Hong Kong, China) for planning the study, and Anna Ringauf (MacroArray Diagnostics) for measuring the samples.

Clinical implications: Multiplex IgE testing including a CCD blocker revealed marked differences in the relevance of CR as primary sensitizer between cohorts from a coastal Asian region and a central European region.

REFERENCES

1. Kang B. Study on cockroach antigen as a probable causative agent in bronchial asthma. J Allergy Clin Immunol 1976;58:357-65.
2. Donfack J, Tsalenko A, Hoki DM, Parry R, Solway J, Lester LA, et al. HLA-DRB1*01 alleles are associated with sensitization to cockroach allergens. J Allergy Clin Immunol 2000;105:960-6.
3. Tripathi P, Hong X, Caruso D, Gao P, Wang X. Genetic determinants in the development of sensitization to environmental allergens in early childhood. Immun Inflamm Dis 2014;2:193-204.
4. Gao P. Sensitization to cockroach allergen: immune regulation and genetic determinants. Clin Dev Immunol 2012;2012:563760.
5. Zhang C, Gjesing B, Lai X, Li J, Spangfort MD, Zhong N. Indoor allergen levels in Guangzhou city, southern China. Allergy 2011;66:186-91.
6. Leung TF, Wong YS, Chan IH, Yung E, Sy HY, Lam CW, et al. Domestic exposure to aeroallergens in Hong Kong families with asthmatic children. Pediatr Pulmonol 2011;46:632-9.
7. Luo W, Hu H, Tang W, Zou X, Huang H, Huang Z, et al. Allergen sensitization pattern of allergic adults and children in southern China: a survey based on real life data. Allergy Asthma Clin Immunol 2019;15:42.
8. Luo W, Chen H, Wu Z, Hu H, Tang W, Chen H, et al. A new trend in sensitization to cockroach allergen: a cross-sectional study of indoor allergens and food allergens in the inland region of Southwest China. Asian Pac J Allergy Immunol 2020 Jun 21. [Epub ahead of print.] https://doi.org/10.12932/AP-281019-0678.
9. Liao C, Hu H, Huang Z, Lin Q, Huang H, Liu X, et al. Shrimp and cockroach co-sensitization in southern China: association with moth sensitization. Allergy Asthma Proc 2020;41:e54-60.
10. Sun BQ, Lai XX, Gjesing B, Spangfort MD, Zhong NS. Prevalence of sensitivity to cockroach allergens and IgE cross-reactivity between cockroach and house dust mite allergens in Chinese patients with allergic rhinitis and asthma. Chin Med J (Engl) 2010;123:3540-4.
11. Liccardi G, Baldi G, Ciccarelli A, Cutajar M, D'Amato M, Gargano D, et al. Sensitization to cockroach allergens in the urban atopic populations living in Campania district (southern Italy). A multicenter study. Eur Ann Allergy Clin Immunol 2014;46:12-6.
12. Lødrup Carlsen KC, Carlsen KH, Buchmann MS, Wikstrøm J, Mehl R. Cockroach sensitivity in Norway: a previously unidentified problem? Allergy 2002;57:529-33.
13. van Wijnen JH, Verhoeff AP, Mulder-Folkerts DK, Brachel HJ, Schou C. Cockroach allergen in house dust. Allergy 1997;52:460-4.
14. Stelmach I, Brzozowska A, Jerzyńska J, Podsiadłowicz-Borzecka M, Bobrowska M, Majak P, et al. [Hypersensitivity to cockroach allergen among children with bronchial asthma living in the Łódź district]. Polski Merkuriusz Lekarski 2000;9:657-61.
15. Hirsch T, Stappenbeck C, Neumeister V, Weiland SK, Von Mutius E, Keil U, et al. Exposure and allergic sensitization to cockroach allergen in East Germany. Clin Exp Allergy 2000;30:529-37.
16. Macan J, Plavec D, Kanceljak B, Milkovic-Kraus S. Exposure levels and skin reactivity to German cockroach (*Blattella germanica*) in Croatia. Croat Med J 2003;44:756-60.

17. Do DC, Zhao Y, Gao P. Cockroach allergen exposure and risk of asthma. Allergy 2016;71:463-74.
18. Rosenstreich DL, Eggleston P, Kattan M, Baker D, Slavin RG, Gergen P, et al. The role of cockroach allergy and exposure to cockroach allergen in causing morbidity among inner-city children with asthma. N Engl J Med 1997;336:1356-63.
19. Arruda LK, Barbosa MC, Santos AB, Moreno AS, Chapman MD, Pomés A. Recombinant allergens for diagnosis of cockroach allergy. Curr Allergy Asthma Rep 2014;14:428.
20. Togias A, Fenton MJ, Gergen PJ, Rotrosen D, Fauci AS. Asthma in the inner city: the perspective of the National Institute of Allergy and Infectious Diseases. J Allergy Clin Immunol 2010;125:540-4.
21. Finn PW, Boudreau JO, He H, Wang Y, Chapman MD, Vincent C, et al. Children at risk for asthma: home allergen levels, lymphocyte proliferation, and wheeze. J Allergy Clin Immunol 2000;105:933-42.
22. Twarog FJ, Picone FJ, Strunk RS, So J, Colten HR. Immediate hypersensitivity to cockroach. Isolation and purification of the major antigens. J Allergy Clin Immunol 1977;59:154-60.
23. Arruda LK, Vailes LD, Mann BJ, Shannon J, Fox JW, Vedvick TS, et al. Molecular cloning of a major cockroach (*Blattella germanica*) allergen, Bla g 2. Sequence homology to the aspartic proteases. J Biol Chem 1995;270:19563-8.
24. Arruda LK, Vailes LD, Hayden ML, Benjamin DC, Chapman MD. Cloning of cockroach allergen, Bla g 4, identifies ligand binding proteins (or calycins) as a cause of IgE antibody responses. J Biol Chem 1995;270:31196-201.
25. Asturias JA, Gómez-Bayón N, Arilla MC, Martínez A, Palacios R, Sánchez-Gascón F, et al. Molecular characterization of American cockroach tropomyosin (*Periplaneta americana* allergen 7), a cross-reactive allergen. J Immunol 1999;162:4342-8.
26. Melén E, Pomés A, Vailes LD, Arruda LK, Chapman MD. Molecular cloning of Per a 1 and definition of the cross-reactive group 1 cockroach allergens. J Allergy Clin Immunol 1999;103:859-64.
27. Binder M, Mahler V, Hayek B, Sperr WR, Scholler M, Prozell S, et al. Molecular and immunological characterization of arginine kinase from the Indianmeal moth, *Plodia interpunctella,* a novel cross-reactive invertebrate pan-allergen. J Immunol 2001;167:5470-7.
28. Pomés A, Wünschmann S, Hindley J, Vailes LD, Chapman MD. Cockroach allergens: function, structure and allergenicity. Protein Peptide Lett 2007;14:960-9.
29. Valenta R, Karaulov A, Niederberger V, Gattinger P, van Hage M, Flicker S, et al. Molecular aspects of allergens and allergy. Adv Immunol 2018;138:195-256.
30. Satinover SM, Reefer AJ, Pomes A, Chapman MD, Platts-Mills TA, Woodfolk JA. Specific IgE and IgG antibody-binding patterns to recombinant cockroach allergens. J Allergy Clin Immunol 2005;115:803-9.
31. Panzner P, Vachova M, Vlas T, Vitovcova P, Brodska P, Maly M. Cross-sectional study on sensitization to mite and cockroach allergen components in allergy patients in the Central European region. Clin Transl Allergy 2018;8:19.
32. Glesner J, Filep S, Vailes LD, Wünschmann S, Chapman MD, Birrueta G, et al. Allergen content in German cockroach extracts and sensitization profiles to a new expanded set of cockroach allergens determine *in vitro* extract potency for IgE reactivity. J Allergy Clin Immunol 2019;143:1474-81.e8.
33. Wang J, Calatroni A, Visness CM, Sampson HA. Correlation of specific IgE to shrimp with cockroach and dust mite exposure and sensitization in an inner-city population. J Allergy Clin Immunol 2011;128:834-7.
34. Celakovska J, Vankova R, Bukac J, Cermakova E, Andrys C, Krejsek J. Atopic dermatitis and sensitisation to molecular components of *Alternaria, Cladosporium, Penicillium, Aspergillus,* and *Malassezia*—results of Allergy Explorer ALEX^2. J Fungi (Basel) 2021;7:183.
35. Bousquet J, Reid J, van Weel C, Baena Cagnani C, Canonica GW, Demoly P, et al. Allergic rhinitis management pocket reference 2008. Allergy 2008;63:990-6.
36. Bousquet J, Clark TJ, Hurd S, Khaltaev N, Lenfant C, O'Byrne P, et al. GINA guidelines on asthma and beyond. Allergy 2007;62:102-12.
37. Ansotegui IJ, Melioli G, Canonica GW, Caraballo L, Villa E, Ebisawa M, et al. IgE allergy diagnostics and other relevant tests in allergy; a World Allergy Organization position paper. World Allergy Organ J 2020;13:100080.
38. Bojcukova J, Vlas T, Forstenlechner P, Panzner P. Comparison of two multiplex arrays in the diagnostics of allergy. Clin Transl Allergy 2019;9:31.
39. Pollart SM, Mullins DE, Vailes LD, Hayden ML, Platts-Mills TA, Sutherland WM, et al. Identification, quantitation, and purification of cockroach allergens using monoclonal antibodies. J Allergy Clin Immunol 1991;87:511-21.
40. Pomes A, Glesner J, Calatroni A, Visness CM, Wood RA, O'Connor GT, 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;144:935-44.
41. Aberer W, Holzweber F, Hemmer W, Koch L, Bokanovic D, Fellner W, et al. Inhibition of cross-reactive carbohydrate determinants (CCDs) enhances the accuracy of *in vitro* allergy diagnosis. Allergol Select 2017;1:141-9.
42. Hemmer W, Altmann F, Holzweber F, Gruber C, Wantke F, Wöhrl S. ImmunoCAP cellulose displays cross-reactive carbohydrate determinant (CCD) epitopes and can cause false-positive test results in patients with high anti-CCD IgE antibody levels. J Allergy Clin Immunol 2018;141:372-81, e3.
43. Adalsteinsdottir B, Sigurdardottir ST, Gislason T, Kristensen B, Gislason D. What characterizes house dust mite sensitive individuals in a house dust mite free community in Reykjavik, Iceland? Allergol Int 2007;56:51-6.
44. Hoffmann-Sommergruber K. Rare food allergens. Allergol Select 2021;5:29-32.
45. Ayuso R, Reese G, Leong-Kee S, Plante M, Lehrer SB. Molecular basis of arthropod cross-reactivity: IgE-binding cross-reactive epitopes of shrimp, house dust mite and cockroach tropomyosins. Int Arch Allergy Immunol 2002;129:38-48.
46. Wong L, Huang CH, Lee BW. Shellfish and house dust mite allergies: is the link tropomyosin? Allergy Asthma Immunol Res 2016;8:101-6.
47. Ukleja-Sokołowska N, Gawrońska-Ukleja E, Lis K, Żbikowska-Gotz M, Adamczak R, Sokołowski Ł, et al. Shrimp sensitization in house dust mite allergic patients. Int J Immunopathol Pharmacol 2020;34:2058738420907188.
48. Klaewsongkram J. High prevalence of shellfish and house dust mite allergies in Asia-Pacific: probably not just a coincidence. Asian Pac J Allergy Immunol 2012;30:247-8.
49. Brown AE, France RM, Grossman SH. Purification and characterization of arginine kinase from the American cockroach (*Periplaneta americana*). Arch Insect Biochem Physiol 2004;56:51-60.
50. García-Orozco KD, Aispuro-Hernández E, Yepiz-Plascencia G, Calderón-de-la-Barca AM, Sotelo-Mundo RR. Molecular characterization of arginine kinase, an allergen from the shrimp *Litopenaeus vannamei*. Int Arch Allergy Immunol 2007;144:23-8.
51. Sarzsinszky E, Lupinek C, Vrtala S, Huang HJ, Hofer G, Keller W, et al. Expression in *Escherichia coli* and purification of folded rDer p 20, the arginine kinase from *Dermatophagoides pteronyssinus:* a possible biomarker for allergic asthma. Allergy Asthma Immunol Res 2021;13:154-63.
52. López-Matas MA, de Larramendi CH, Moya R, Sánchez-Guerrero I, Ferrer A, Huertas AJ, et al. *In vivo* diagnosis with purified tropomyosin in mite and shellfish allergic patients. Ann Allergy Asthma Immunol 2016;116:538-43.
53. Giuffrida MG, Villalta D, Mistrello G, Amato S, Asero R. Shrimp allergy beyond tropomyosin in Italy: clinical relevance of arginine kinase, sarcoplasmic calcium binding protein and hemocyanin. Eur Ann Allergy Clin Immunol 2014;46:172-7.
54. Pedrosa M, Boyano-Martínez T, García-Ara C, Quirce S. Shellfish allergy: a comprehensive review. Clin Rev Allergy Immunol 2015;49:203-16.
55. Ruethers T, Taki AC, Johnston EB, Nugraha R, Le TTK, Kalic T, et al. Seafood allergy: a comprehensive review of fish and shellfish allergens. Mol Immunol 2018;100:28-57.
56. Gelis S, Rueda M, Valero A, Fernández EA, Moran M, Fernández-Caldas E. Shellfish allergy: unmet needs in diagnosis and treatment. J Investig Allergol Clin Immunol 2020;30:409-20.
57. Matsui EC, Sampson HA, Bahnson HT, Gruchalla RS, Pongracic JA, Teach SJ, et al. Allergen-specific IgE as a biomarker of exposure plus sensitization in inner-city adolescents with asthma. Allergy 2010;65:1414-22.