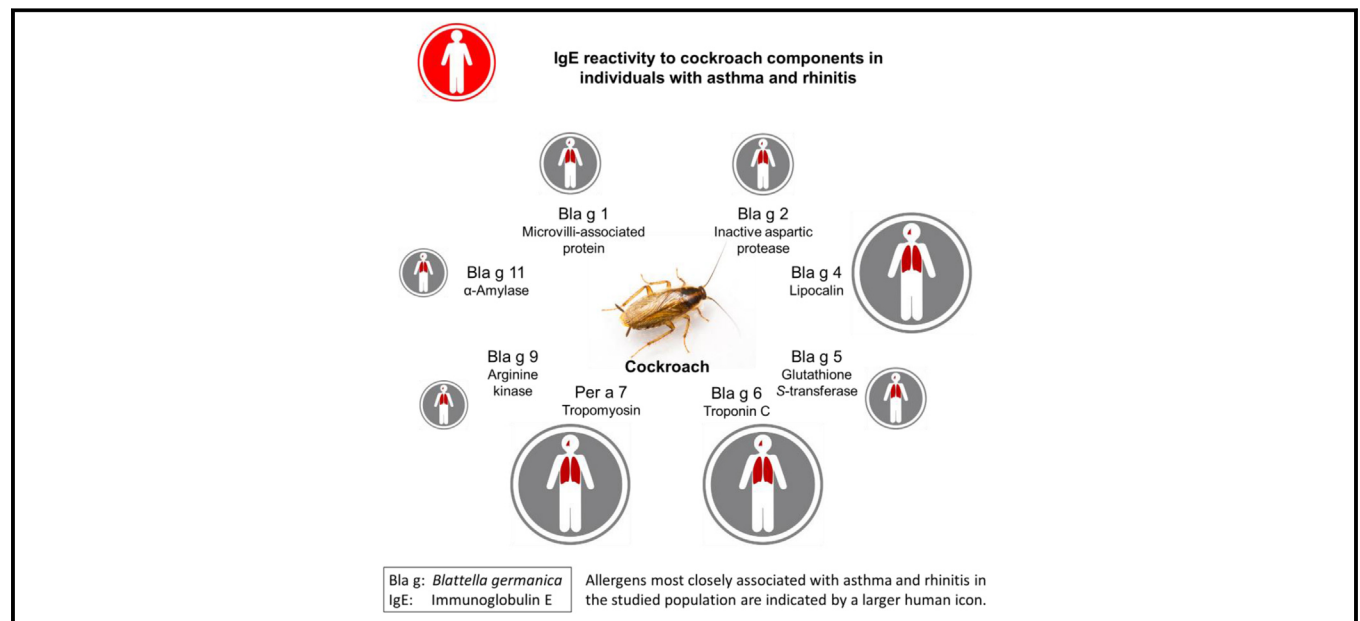


Cockroach allergen component analysis of children with or without asthma and rhinitis in an inner-city birth cohort

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GRAPHICAL ABSTRACT



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Background: Cockroach is one of the most important sources of indoor allergens and can lead to IgE sensitization and development of rhinitis and asthma.

Objective: We sought to perform a cockroach allergen component analysis to determine the allergens and antibody levels and patterns of sensitization associated with asthma and rhinitis.

Methods: Antibody (IgE, IgG, and IgG₄) levels to total cockroach and 8 cockroach allergens were determined in 2 groups of cockroach-sensitized 10-year-old children with (n = 19) or without (n = 28) asthma and rhinitis. Allergen-specific antibody levels were measured in streptavidin ImmunoCAPs loaded with each of the recombinant allergens from groups 1, 2, 4, 5, 6, 7, 9, and 11, and total cockroach-specific IgE levels were measured with the i6 ImmunoCAP.

Results: IgE antibody levels to cockroach allergens and extract, but not IgG or IgG₄ antibody levels, differed between subjects with and without asthma and rhinitis. Specifically, recognition of more cockroach allergens with higher allergen-specific IgE levels was associated with disease. Variable patterns of sensitization with no immunodominant allergens were found in both groups. There was a good correlation between the sum of allergen-specific IgE and total cockroach IgE levels ($r = 0.86$, $P < .001$).

Conclusions: Component analysis of 8 cockroach allergens revealed significant differences in IgE reactivity associated with the presence of asthma and rhinitis. Allergen-specific IgE titers and sensitization profiles were associated with asthma and rhinitis. (J Allergy Clin Immunol 2019;■■■:■■■-■■■.)

Key words: Cockroach allergy, cockroach allergen components, asthma, rhinitis, diagnosis, immunotherapy

Cockroach allergy is an important health problem that can lead to the development of asthma, rhinitis, or both. In the United States the main species of cockroach that causes allergy is the German cockroach *Blattella germanica*. Exposure and sensitization to cockroach allergens are 2 factors strongly associated with high morbidity among inner-city children with asthma.¹ The profile of IgE sensitization to cockroach allergens is unique for each patient.^{2,3} Unlike allergies to cat or mite, which are mostly associated with sensitization to the major allergens Fel d 1 or Der p 1 and Der p 2, respectively, no immunodominant allergens have been found for cockroach. Bla g 2 has been considered the major cockroach allergen in the United States, followed by Bla g 5, with prevalences of IgE sensitization of 42% to 70% and 35% to 68%, respectively.^{2,4,5} In the last 10 years, new allergens have emerged from cockroach and have been classified into 13 groups currently listed in the allergen nomenclature database of the World Health Organization and International Union of Immunological Societies (www.allergen.org). However, the relevance of sensitization to a large set of cockroach allergens for disease has not been deeply investigated.

In the current study sensitization to 8 groups of cockroach allergens was analyzed among cockroach-sensitized patients with disease (asthma and rhinitis) or without disease. The cockroach allergen groups are as follows: group 1, gut microvilli-associated proteins; group 2, gut inactive aspartic proteases; group 4, lipocalins produced only in males and excreted in the spermatophore during copulation; group 5, glutathione S-transferases;

Abbreviation used

URECA: Urban Environment and Childhood Asthma

group 6, troponin C, which is involved in muscle contraction; group 7, tropomyosins; group 9, arginine kinases; and group 11, α -amylases.⁴⁻¹⁷ These allergens were selected based on their importance, which has been recently described.³

One of the major goals of the Inner-City Asthma Consortium sponsored by the National Institute of Allergy and Infectious Diseases is to develop an immune-based therapeutic approach to asthma by targeting cockroach allergy.¹⁸ It is known that the allergen content of Bla g 1 and Bla g 2 in cockroach extracts is variable.^{19,20} Recently, up to 20-, 728-, and 12-fold differences for Bla g 1, Bla g 2, and Bla g 5, respectively, have been reported in cockroach extracts.³ The potency of the extracts was found to be dependent not only on the allergen content of the extract but also on the subject sensitization profile.³ Additionally, new major allergens were identified in a small group of subjects in the United States.³ It is essential to evaluate sensitization patterns to this new large set of cockroach allergens to assess their relevance to disease.

In this study a component analysis of the antibody reactivity (IgE, IgG, and IgG₄) to 8 cockroach allergens was performed in 2 groups of cockroach-sensitized children participating in the Inner-City Asthma Consortium's Urban Environment and Childhood Asthma (URECA) birth cohort study. One group included subjects with asthma and rhinitis, whereas the subjects in the other group did not have these diseases. The goal was to identify the main cockroach allergens and to evaluate differences in allergen-specific patterns of sensitization between subjects with and without asthma and rhinitis. The analysis performed here is important for designing clinical trials and ensuring that patients are treated with cockroach extracts that contain allergens relevant to their disease.

METHODS

Study population

The URECA birth cohort has been previously described.²¹ This observational birth cohort of 560 infants was studied after recruitment of pregnant mothers from 4 research centers (Johns Hopkins University, Baltimore, Maryland; Boston University, Boston, Massachusetts; Columbia University and Mt Sinai University, New York City, New York; and Washington University, St Louis, Missouri). Inclusion criteria were as follows: residence in a census tract with at least 20% of residents below the poverty level; at least 1 parent with a history of allergic rhinitis, eczema, and/or asthma; and birth of the enrolled child at 34 weeks' gestation or later. The Human Subjects Committees at the University of Wisconsin and the 4 clinical sites approved the study. The total number of URECA cohort participants at age 10 years, when the samples were obtained for the current study, was 442 children, the same number as at 7 years of age.²²

The population selected consisted of cockroach-sensitized 10-year-old children from the URECA cohort with either positive cockroach-specific IgE levels as determined based on streptavidin ImmunoCAP results, positive cockroach-specific skin prick test responses, or both. These 78 cockroach-sensitized patients were subdivided into 4 groups: (1) no asthma and no rhinitis (28/78 [35.9%]), (2) rhinitis only (18/78 [23.1%]), (3) asthma only (13/78 [16.7%]), and (4) asthma and rhinitis (19/78 [24.4%]). Groups 1 and 4 were selected for component analysis (n = 47) because their most different phenotypes were expected to facilitate the observation of differences in

component analysis. They were divided into 2 groups: children with rhinitis and asthma ($n = 19$) and children without disease ($n = 28$). Classification of asthma (asthma symptoms or asthma medication use in the prior year) and rhinitis (presence of runny and/or stuffy nose and frequent sneezes in the prior year) for the purpose of sample selection was determined based on parental report. Twelve of the 19 children selected on the basis of reported symptoms or medication use also met the more strict criteria for asthma, as defined by O'Connor et al.²³ Additional evidence of recent activity at age 10 years (lung function testing; symptoms, including those in the past 12 months; and medication use) is shown in Table I.

Patients of the same age who were not allergic to cockroach (but exposed to at least 2 units of Bla g 1 [208 ng/g] and Bla g 2 [80 ng/g]) were used as negative control subjects ($n = 10$). Positive exposure to cockroach allergens for negative control subjects was selected to ensure that the patients were not allergic to cockroach.

Expression, purification, and quantification of 8 cockroach allergens

The German cockroach allergens Bla g 1, Bla g 2, Bla g 4, Bla g 6, Bla g 9, and Bla g 11 and the American cockroach allergen Per a 7.0102 (>98% amino acid identity to Bla g 7) were expressed in *Pichia pastoris*, and Bla g 5 was expressed in *Escherichia coli*. All the allergens were expressed and purified as described in the Methods section in this article's Online Repository at www.jacionline.org.

Measurement of IgE, IgG, and IgG₄ antibody levels

Total cockroach-specific and cockroach allergen-specific IgE, IgG, and IgG₄ antibody levels were measured in sera by using i6 ImmunoCAPs (commercially available CAPs loaded with cockroach extract) and in-house allergen-loaded streptavidin ImmunoCAPs, respectively, in a Thermo Fisher Scientific ImmunoCAP system (Phadia 250 Immunoassay Analyzer; Thermo Fisher Scientific, Portage, Mich).

Before measuring allergen-specific antibody levels, several steps were performed to optimize the assays that include allergen biotinylation, optimization of biotinylation, optimization of the amount of biotinylated allergen loaded into the streptavidin ImmunoCAPs, stability analysis of allergen-loaded ImmunoCAPs, and evaluation of the specificity of the assay, as explained in the Methods section in this article's Online Repository. Biotinylated allergens were loaded and incubated on streptavidin ImmunoCAPs by using the Phadia 100. Assays for IgE, IgG, and IgG₄ measurements were performed according to the manufacturer's instructions. The lower limits of quantification were 0.1 kU_A/L for IgE, 2 mg/L for IgG, and 0.07 mg/L for IgG₄. A conservative value of 0.35 kU_A/L or greater was specified as the cutoff value for a positive allergen-specific IgE response to calculate prevalences of IgE sensitization. IgE antibodies specific to other allergen sources were also measured by using the ImmunoCAP system with commercially available ImmunoCAPs loaded with extracts (*Aspergillus* species, *Alternaria* species, mouse, timothy grass, oak, ragweed, cat, maple, dog, and mite [*Dermatophagoides pteronyssinus* and *Dermatophagoides farinae*]).

Statistical analysis

Antibody levels (IgE, IgG, and IgG₄) and total IgE values were right skewed based on a diagnostic plot and the results of a Shapiro-Wilk test and were therefore log-transformed for statistical tests. The results of these tests are presented as geometric means and geometric SDs, and the comparisons between groups are presented as the ratio, 95% CI, and P value for testing the effect with respect to the group comparison.

Fisher exact or χ^2 tests were used for categorical variables, and t tests or Wilcoxon tests were used for normally distributed and nonnormally distributed continuous variables, respectively, to compare differences in baseline characteristics and demographics between no disease and asthma and rhinitis.

TABLE I. Description of the population

	No disease (n = 28)	Asthma and rhinitis (n = 19)	P value
Site			.56
Baltimore	6 (21.4%)	4 (21.1%)	
Boston	6 (21.4%)	5 (26.3%)	
New York	11 (39.3%)	4 (21.1%)	
St Louis	5 (17.9%)	6 (31.6%)	
Mother's race			.69
Black	19 (67.9%)	13 (68.4%)	
Hispanic	8 (28.6%)	4 (21.1%)	
White	1 (3.6%)	1 (5.3%)	
Other	0 (0.0%)	1 (5.3%)	
Mother atopic? Yes	25 (89.3%)	17 (89.5%)	.99
Mother had asthma? Yes	14 (50.0%)	14 (73.7%)	.19
Mother ever had asthma?	11 (39.3%)	13 (68.4%)	.10
Yes			
Mother had eczema? Yes	11 (39.3%)	7 (36.8%)	.99
Child's race			.61
Black	19 (67.9%)	13 (68.4%)	
Hispanic	8 (28.6%)	4 (21.1%)	
White	1 (3.6%)	2 (10.5%)	
Child's sex			.63
Female	12 (42.9%)	6 (31.6%)	
Male	16 (57.1%)	13 (68.4%)	
Child's characteristics at 10 y			
FEV ₁ /FVC ratio	0.84 (0.82-0.88)	0.81 (0.71-0.85)	.08
FEV ₁ (% predicted)	106 (94.7-114)	101 (87.0-107)	.08
Blood eosinophil count	200 (100-200)	500 (325-700)	<.01
BMI percentile	84.1 (59.5-99.1)	85.6 (46.9-99.8)	.75
Any wheeze by report or doctor's office visit? Yes	1 (3.6%)	11 (57.9%)	<.01
Report of eczema or EASI score ≥ 1 ? Yes	5 (17.9%)	5 (26.3%)	.50
Inhaled steroid? Yes	0 (0.0%)	8 (42.1%)	<.01
Oral steroid? Yes	4 (16.0%)	9 (47.4%)	.05

Comparison of groups with asthma and rhinitis and no disease from the URECA cohort for demographic variables. Summary statistics are frequencies (percentages) for categorical variables and medians (first-third quartiles) for continuous variables with comparison between groups using χ^2 test or Wilcoxon tests, respectively. BMI, Body mass index; FVC, forced vital capacity; EASI, Eczema Area and Severity Index

Relative importance was calculated by using the *relaimpo* R package with the "lmg" method.²⁴ The R^2 value of a model represents the proportion of variance explained by the set of predictors included in the model. The "lmg" method calculates the relative and independent contribution of each predictor to the R^2 value while taking care of dependence on the ordering of predictors within the model.

Because of the exploratory nature of the analysis, no adjustments or no attempts were made to adjust for multiple comparisons. The level of significance was set at a P value of less than .05. All analyses were performed with R software (version 3.5), and figures were constructed with the R *lattice* package.

RESULTS

Description of the patient population

Subjects were 10-year-old children from low-income neighborhoods in urban Baltimore, Boston, New York, and St Louis. Most of the children were black (approximately 68%), followed by Hispanic (21% to 29%) and white (3% to 11%), with similar

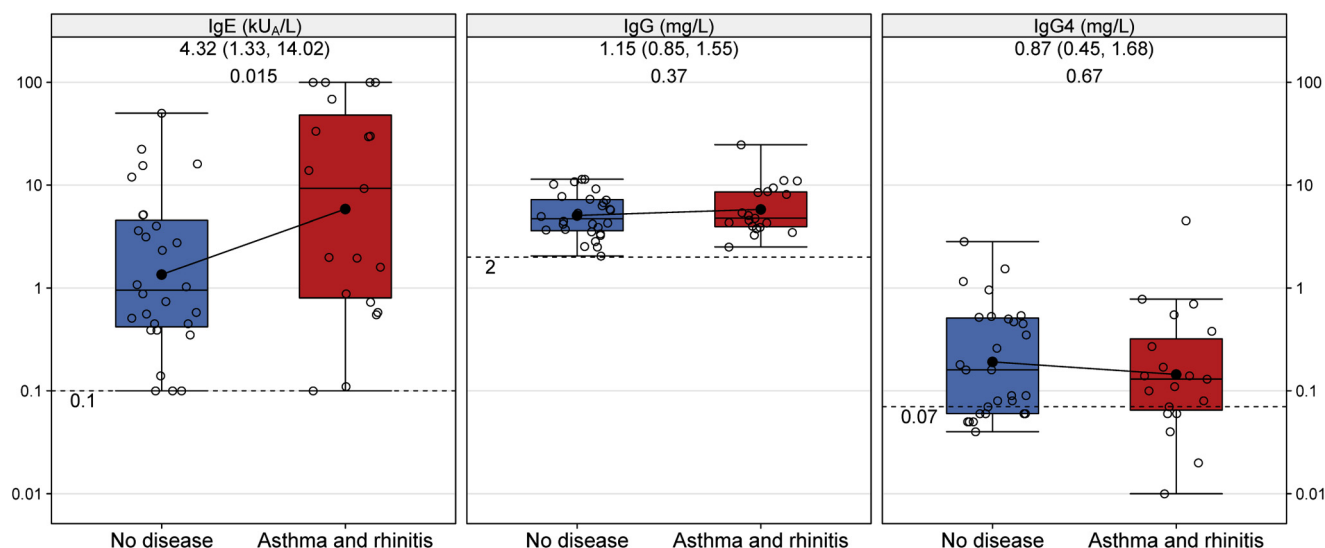


FIG 1. Comparison of cockroach-specific IgE, IgG, and IgG₄ antibody levels in subjects from groups with no disease and asthma and rhinitis. In the box plots the solid horizontal line and filled circle within a box represent the median and geometric mean, respectively; the box margins are the interquartile range (50% of the observations); whisker lines extend for 1.5 times the interquartile range; and individual observations are marked with a hollow circle. Dashed lines represent cutoff values of quantification (values under the cutoff for IgG and IgG₄ were included for plotting purposes, and IgE values under the cutoff were plotted as 0.1 kU_A/L). The annotations in each panel are ratios between groups (95% CI) and *P* values.

percentages for their mothers (Table I). URECA is a high-risk birth cohort, and therefore a large proportion of the 47 tested subjects' mothers were atopic (approximately 89%) and had asthma (50% to 74%) or eczema (36% to 40%). The 2 groups of patients with cockroach allergy analyzed were similar in terms of maternal atopy (atopy, asthma, and eczema) and the child's race and sex, but significant differences were found regarding blood eosinophil counts, wheeze by report or doctor's office visit, and use of inhaled steroids (Table I).

Differences of IgE sensitization to allergen sources between groups with and without disease

Subjects were sensitized to other allergen sources in addition to cockroach. The sum of 12 allergen source-specific IgEs was only about 3% of total IgE, and this proportion was similar for the group without disease (2.8%) and the rhinitis and asthma group (4.3%, *P* = .23; see Fig E1 in this article's Online Repository at www.jacionline.org). On the other hand, the correlation between the sum of 12 allergen source-specific IgE and total IgE levels was high (*r* = 0.82, *P* < .001), whereas the correlation between cockroach-specific IgE and total IgE levels was moderate (*r* = 0.64, *P* < .001; see Fig E2 in this article's Online Repository at www.jacionline.org). The rhinitis and asthma group were more highly sensitized based on several metrics, including specific IgE, and based on skin prick test responses to *Alternaria* species, mouse, ragweed, and mite (*D pteronyssinus*; *P* ≤ .05); the number of positive skin test results to aeroallergens (*P* = .02); the sum of specific IgE levels (*P* = .01); and the number of positive specific IgE levels (*P* = .01; see Table E1 in this article's Online Repository at www.jacionline.org). There were no significant differences in the proportion (percentage) of specific IgEs to 12 different sources versus the sum of 12 specific IgE levels between the 2 groups (data not shown).

TABLE II. Levels of sensitization to cockroach for subjects studied

CAP class	IgE (kU _A /L)	Interpretation	No. of patients*		Total
			No disease	Asthma and rhinitis	
0	<0.35	Undetectable	4	2	6
1	0.35 to <0.7	Equivocal	8	2	10
2	0.7 to <3.5	Positive	7	5	12
3	3.5 to <17.5	Positive	7	2	9
4	17.5 to <50	Strong Positive	1	3	4
5	50 to <100	Strong Positive	1	1	2
6	≥100	Strong Positive	0	4	4
Total			28	19	47

**P* = .01, χ^2 trend test.

IgE, IgG, and IgG₄ antibody levels to cockroach extract

Statistically significant differences between groups with and without disease were found for cockroach-specific IgE (ratio, 4.32 [95% CI, 1.33-14.02]; *P* = .015) but not for IgG and IgG₄ (ratio, 1.15 [95% CI, 0.85-1.55; *P* = .37] and 0.87 [95% CI, 0.45-1.68; *P* = .67], respectively; Fig 1 and see Table E2 in this article's Online Repository at www.jacionline.org). Antibody levels for groups without and with disease were 1.35 versus 5.85 kU_A/L for IgE (4.3-fold), 5.06 versus 5.80 mg/L for IgG (1.1-fold), and 0.21 versus 0.18 mg/L for IgG₄ (0.8-fold), respectively (see Table E2). IgG₄ antibody levels to cockroach extract were low (with 28% [16/57] of subjects at less than the 0.07 mg/L cutoff) and contributed only 6 ± 7% to IgG antibody levels. The 3 correlations between 2 of the 3 variables were significant (*r* = 0.53 between IgE and IgG, *P* < .001; *r* = 0.35 between IgE and IgG₄,

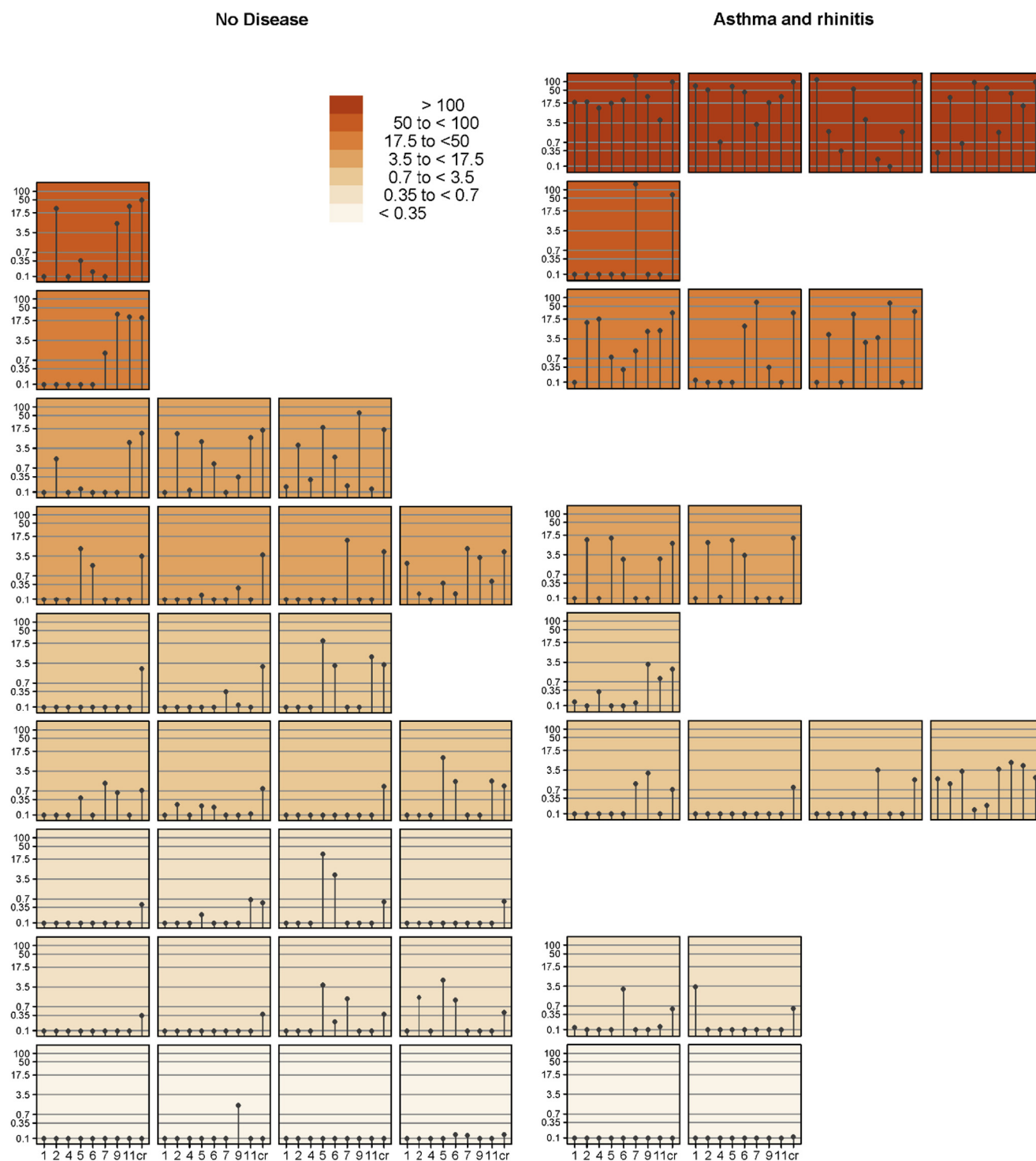


FIG 2. Individual patterns of IgE sensitization to cockroach components and cockroach extract. The no disease (*left*) and asthma and rhinitis (*right*) groups are compared. Allergens are indicated as groups 1, 2, 4, 5, 6, 7, 9, and 11, and cockroach extract is indicated by *cr*. Increasing CAP class ranges are indicated by a graded increase of red tones (from 0-6). Panels are ordered from lowest to highest levels of cockroach-specific IgE.

$P = .017$; and $r = 0.35$ between IgG and IgG₄, $P = .015$; see Fig E3 in this article's Online Repository at www.jacionline.org).

More patients had high IgE titers to cockroach in the group with asthma and rhinitis than in the group without disease (8 patients

belonged to the high CAP classes 4-6 in the group with disease vs only 2 in the group without disease, Table II). Total IgE levels also showed a significant increase between both groups (ratio, 2.36; $P = .039$) but not the cockroach IgE/total IgE ratio (ratio, 2.53;

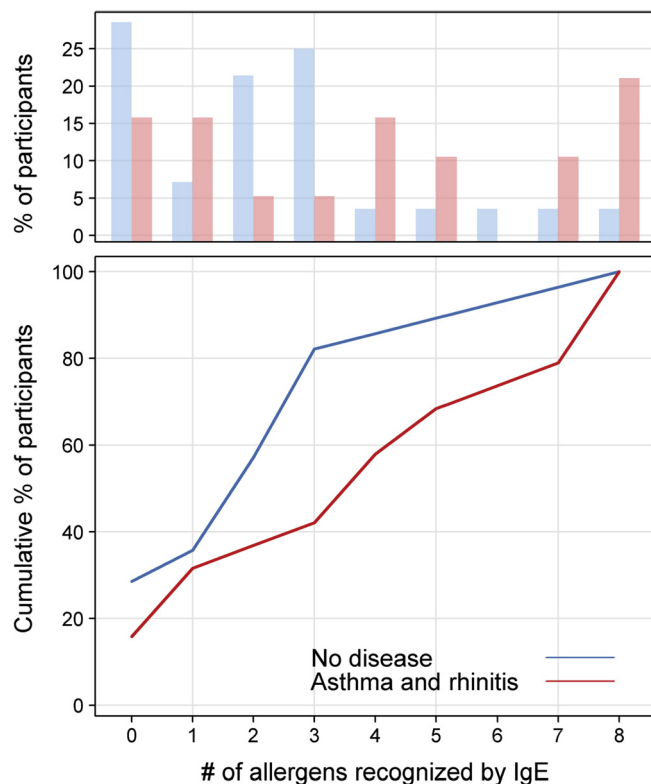


FIG 3. Number of cockroach component allergens recognized by patients with no disease and those with asthma and rhinitis (cutoff of 0.1 kU_A/L).

$P = .064$) and skin prick test wheal size (difference, 0.9; $P = .34$; see Fig E4 in this article's Online Repository at www.jacionline.org).

Individual pattern of IgE sensitization to 8 cockroach allergens

A variable pattern of IgE antibody levels to 8 cockroach allergens was found for all patients (Fig 2). Within each group, a larger cockroach-specific IgE level was associated with sensitization to more allergens and greater allergen-specific IgE antibody titers (Fig 2).

Subjects with disease had greater levels of IgE sensitization and recognized a broader range of allergens (Fig 2). Most patients with disease (79%) recognized up to 7 allergens, and most without disease (82%) recognized up to only 3 allergens (Fig 3). The sum of 8 allergen-specific IgE levels was significantly greater in the group with disease (see Fig E5 in this article's Online Repository at www.jacionline.org).

Component analysis of IgE, IgG, and IgG₄ antibodies to 8 cockroach allergens

Differences in allergen-specific IgE antibody levels between both groups were significant for most allergens tested ($P < .05$), except for Bla g 5 ($P = .33$), Bla g 9 ($P = .076$), and Bla g 11 ($P = .34$; Fig 4 and see Table E2). In contrast, allergen-specific IgG and IgG₄ levels to cockroach component proteins were similar in the 2 groups (see Table E2). Most cockroach component

IgG₄ values were less than the cutoff level of quantification (70 ng/L) and were similarly low in the 2 groups.

There was an excellent correlation between cockroach-specific IgE levels and the sum of 8 allergen-specific IgE levels ($r = 0.86$, $P < .001$; Fig 5). This correlation was better for the group with disease than for those without ($r = 0.97$ vs $r = 0.69$, $P < .001$ for both; see Fig E2).

No significant differences in allergen-specific IgG levels were found between IgE-sensitized subjects and nonsensitized negative control subjects (see Tables E3 and E4 in this article's Online Repository at www.jacionline.org). No significant differences by sex were found for IgE, IgG, or IgG₄ to cockroach components in each of the 2 groups and the negative control subjects (data not shown).

Interestingly, 11 subjects (23% of 47, 8 from the group without disease and 3 from the group with disease) did not react to the 8 cockroach allergens tested.

Among the cockroach-sensitized subjects with disease, Per a 7 was a major allergen (prevalence of IgE sensitization $\geq 50\%$). No major allergen was found for the group of subjects without disease. Per a 7 was the most recognized allergen in the group with disease (10/19 [52.6%]), and Bla g 5 was the most recognized allergen in the group without disease (11/28 [39.3%], see Table E5 in this article's Online Repository at www.jacionline.org). No significant correlation was found between cockroach- and mite (*D pteronyssinus*)-specific IgE levels among the 16 patients who recognized Per a 7. When considering only those participants who were highly sensitized to total cockroach (CAP class ≥ 3), Bla g 5, Bla g 9, and Bla g 11 were major in the group without disease, whereas all except Bla g 1 were major in the group with disease (see Table E5). Bla g 1 was the least recognized allergen in both groups (6/47, see Table E5).

Regarding the proportion of allergen-specific IgEs versus their sum, there was no allergen dominating the response to cockroach (ie, $>50\%$). Bla g 5 showed the largest proportion (approximately 10%), and Bla g 4 showed the lowest proportion ($<5\%$, see Fig E6, top, in this article's Online Repository at www.jacionline.org). There were not statistically significant differences of this variable between the 2 groups, except for Bla g 11 (see Fig E6, bottom).

Relationship of component tests to clinical disease

A relative importance analysis was performed to identify and quantify which IgE antibodies to 8 cockroach allergens were the most closely associated with asthma and rhinitis. The total proportion of variance explained by the model with these biomarkers was 38.4%. Bla g 6, Bla g 4, and Per a 7 were most closely associated with asthma and rhinitis, whereas Bla g 11 and 9 were the least important in explaining disease (Fig 6).

DISCUSSION

Although cockroach allergy is closely associated with respiratory disease in urban centers, the specific features of cockroach sensitization that are associated with disease are unknown. In this study the antibody reactivity to a set of 8 cockroach allergens was evaluated in 2 groups of patients with cockroach allergy from the URECA cohort: those with or without asthma and rhinitis. The main goal was to perform a component analysis to investigate

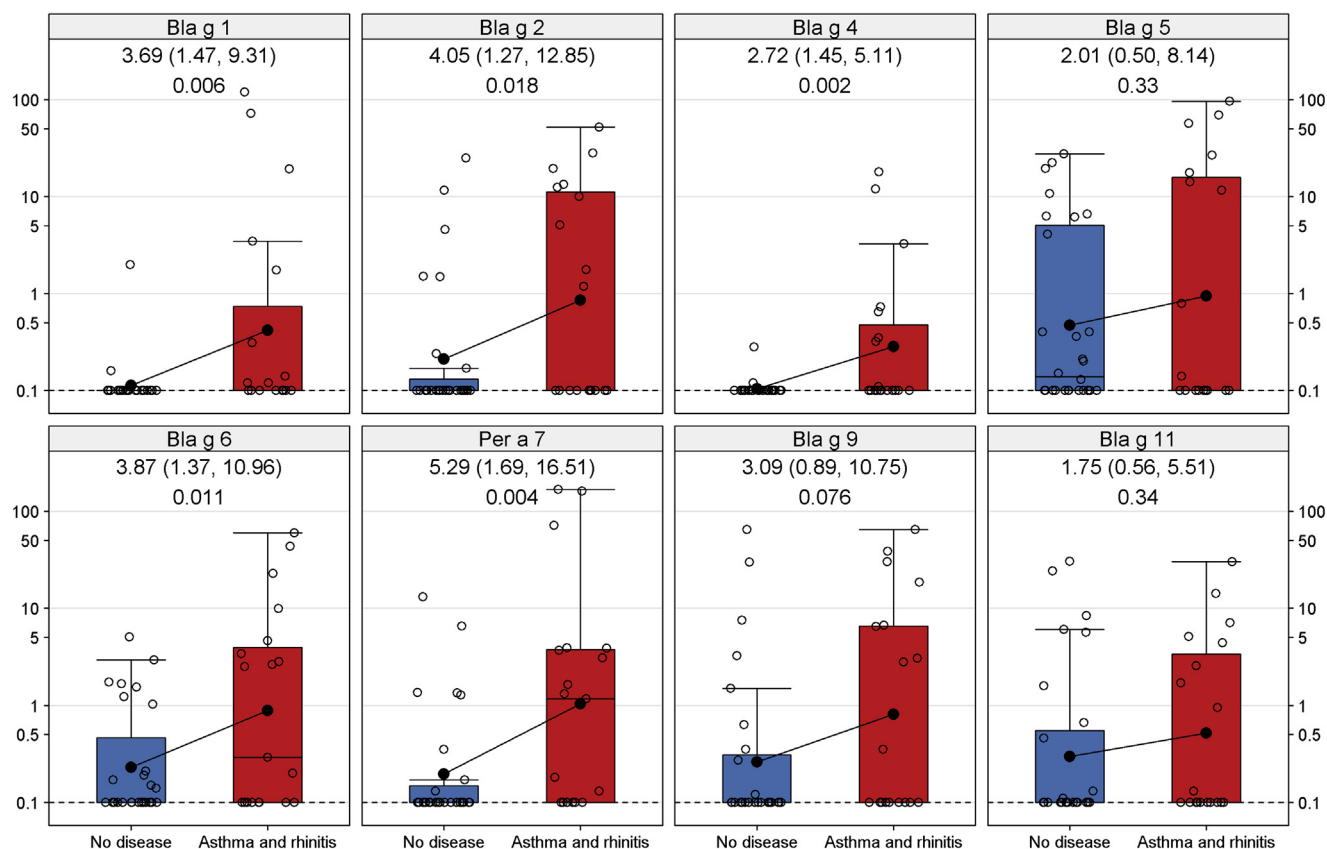


FIG 4. Comparison of IgE reactivity to each cockroach allergen between groups without and with disease. In the box plots the solid horizontal line and filled circle within a box represent the median and geometric mean, respectively; the box margins are the interquartile range (50% of the observations); whisker lines extend for 1.5 times the interquartile range; and individual observations are marked by a hollow circle.

whether specific allergens, levels of sensitization, or both were associated with disease. Levels of IgE, IgG, and IgG₄ antibodies to total cockroach and each of 8 different cockroach allergens from groups 1, 2, 4, 5, 6, 7, 9, and 11 were measured. Reactivity of antibodies developed against cross-reactive allergens from other insect (including cockroach) species might have also been measured (ie, allergens from groups 1 and 7). However, this reactivity is expected to be low because German cockroach is the most common cockroach species in the United States, where the studied population originated. Future studies could analyze cross-reactivity among cockroach allergens in areas such as Florida, where German and American cockroaches coexist. One of the strengths of the study is the multicenter population, and data are representative of populations in 4 large urban areas: New York City, Boston, Baltimore, and St Louis. The demographics (70% African American and 25% Hispanic) are broadly representative of many, but not all, large urban centers.

The main finding was that the patients with cockroach allergy and asthma and rhinitis were overall IgE sensitized to more allergens and at greater levels than the subjects without disease. No differences regarding site, mother's and child's race, and child's sex between the 2 groups accounted for these observations (as previously also reported for race regarding allergic sensitization and asthma severity).^{25,26} Even with a relatively small cohort of subjects in the control and disease groups, the differences found between both groups were significant.

Future studies in larger cohorts will need to address differences between patients with asthma and rhinitis. The previously reported importance of Bla g 2 among cockroach-sensitized patients² was questioned by the results that show additional important allergens (ie, from groups 5, 7, 9, and 11). However, no component-specific IgEs were associated exclusively with the group with disease. Equivalent findings have been reported for only a few other allergen sources. Recognition of a larger number of house dust mite allergens (up to 7) with greater IgE levels was observed in patients with asthma versus nonasthmatic subjects.²⁷ Similar results were found for atopic dermatitis: patients with severe disease had a significantly greater frequency of IgE reactivity to allergens from cat (rFel d 1) and house dust mite (rDer p 4 and rDer p 10) and had an IgE reactivity profile that was more spread toward different allergens compared with patients with moderate atopic dermatitis. On the other hand, there were no significant differences in the frequencies of IgE reactivity to grass pollen allergens between both groups.²⁸ Finally, greater levels of IgE to Fel d 1 were also observed in children with cat allergy with asthma compared with those with rhinoconjunctivitis.²⁹

Differences observed at the level of allergen-specific and cockroach-specific IgE are not reflected in cockroach skin prick test reactivity, which was similar between the 2 groups. In this sense total cockroach IgE levels and component analysis provide an additional level of information about the subject's reactivity.

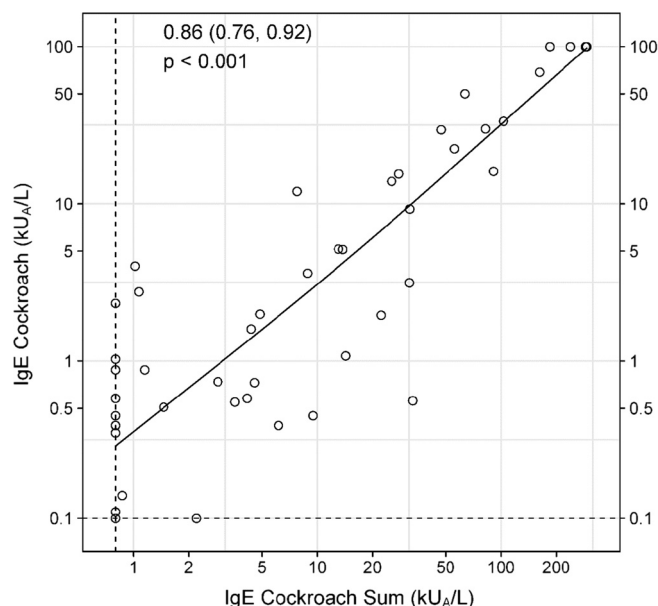


FIG 5. Correlation between cockroach-specific IgE levels and the sum of 8 allergen-specific IgE levels. Annotations in the plot area are Pearson correlations (95% CI) and *P* values.

Total cockroach IgE levels and percentages of total IgE accounted for by cockroach-specific IgE were significantly greater for the group with asthma and rhinitis.

Total IgE levels to perennial allergens have been reported to be associated with asthma.³⁰ The median level of total IgE is typically 200 to 400 kU_A/L in patients with atopic disease.³⁰ The values found here were around this range (197.35 vs 464.86 kU_A/L for the groups without and with disease, respectively). Interestingly, the sum of IgE specific to 12 different allergen sources, including cockroach, was not statistically different for the 2 groups of cockroach-sensitized subjects. The sum of IgE specific to 12 different allergen sources accounted for less than 5% of total IgE. Despite this small proportion, there was an excellent correlation between that sum of source-specific IgE levels and total IgE levels. For the 2 groups, cockroach-specific IgE was the greatest among all the source-specific IgEs tested, followed by *D pteronyssinus* and *D farinae* (all 3 indoor allergen sources). Overall, both groups showed a similar proportion of IgE sensitization to different sources. The magnitude of the sensitization (assessed by IgE titers and the number of allergens to which patients were sensitized) was the main difference between the groups.

The panel of 8 allergens covers most of the IgE reactivity to cockroach extract, as shown by the high correlation between cockroach-specific IgE levels and the sum of the 8 allergen-specific IgE levels. None of the allergens seemed to dominate the response to cockroach because the proportion of IgE to each allergen versus the sum of all cockroach allergen-specific IgE levels was as high as approximately 10% for Bla g 5 and as low as less than 5% for Bla g 4, but none were greater than 50%. This is in contrast with mite or cat allergy, in which a large proportion of the IgE reactivity is directed to immunodominant allergens (ie, anti-Der p 1 and anti-Der p 2 IgE together accounted on average for 85% of the mite-specific IgE).³¹

Some patients with cockroach allergy (11/47 [23%]) did not recognize the 8 allergens tested, which suggests that other proteins are involved in cockroach sensitization. This phenomenon is not unusual and has also been observed for mite allergy in a cohort of patients from which 11.3% (11/97) did not have IgE reactivity to a panel of 13 mite allergens in a microarray.³² Most of the patients with cockroach allergy who did not recognize any allergen had low IgE antibody levels and might be sensitized to additional, most likely minor allergens that were either not tested or yet to be identified. Efforts in this direction to test potentially relevant new cockroach allergens are currently ongoing in our laboratory and could lead to an expanded component test with increased sensitivity versus the current one.

The prevalence of allergen-specific IgE sensitization depended on the total cockroach-specific IgE titers of the subjects chosen. For example, no major allergens (recognized by >50% of patients) were found for the whole population of cockroach-sensitized subjects tested (*n* = 47). In contrast, among a subgroup of highly allergic patients (CAP class > 3), most allergens (all except Bla g 1) were major in the disease group, whereas Bla g 5, Bla g 9, and Bla g 11 were major in the subjects without disease. This lack of immunodominant allergens regarding the prevalence of IgE sensitization highlights an important difference between cockroach and other allergen sources. Patients with mite, cat, and birch allergy are mainly sensitized to only 1 or a few major allergens with high IgE prevalences (Der p 1, Der p 2, and Der p 23 for mite; Fel d 1 for cat; and Bet v 1 for birch). This observation has implications for immunotherapy, which might be effective for more patients when dominant allergens are administered. For example, Pauli et al³³ showed that administration of only rBet v 1 was as effective as administration of birch extracts for the specific treatment of birch pollen allergy. For cockroach, administration of more allergens might be necessary for a vaccine to be effective for more patients.

Only a few studies report associations between IgE sensitization to specific cockroach allergens and disease. In Taiwan a component analysis revealed that 8 American cockroach allergens did not have equal importance in terms of pathogenicity among subjects of a wide range of ages (8-86 years).³⁴ IgE reactivity to allergens from groups 1 to 7 and Per a 9 were compared between patients with asthma and rhinitis or those with rhinitis alone. A high proportion of subjects with persistent asthma and rhinitis (81% [17/21]) had IgE reactivity to Per a 2. In contrast, 80% (16/20) of patients with only allergic rhinitis had IgE reactivity to Per a 9 compared with only 29% (6/21) of patients with asthma and rhinitis. The greatest prevalences of IgE sensitization in patients with asthma and rhinitis were for Per a 2 (81%), followed by Per a 5 (67%). These 2 allergens also dominated the IgE antibody responses to 5 cockroach allergens in a US cohort of 118 patients highly allergic to cockroach. Among sera with high IgE levels to cockroach extract (3.5-100 IU/mL), the prevalence of IgE antibodies to Bla g 2 and Bla g 5 were the greatest (71% and 58%, respectively).²

Unlike the Taiwanese study, groups of 10-year-old children with and without asthma and rhinitis were compared here. The prevalence of IgE sensitization to any 1 specific allergen was less than 40% in the patients with cockroach allergy without disease, which is in contrast to a greater prevalence in the group with disease (>40% for most allergens except Bla g 1 and Bla g 4 by using a conservative cutoff of 0.35 kU_A/L instead of 0.1 kU_A/L). Among highly allergic patients with disease (CAP ≥ 3), IgE

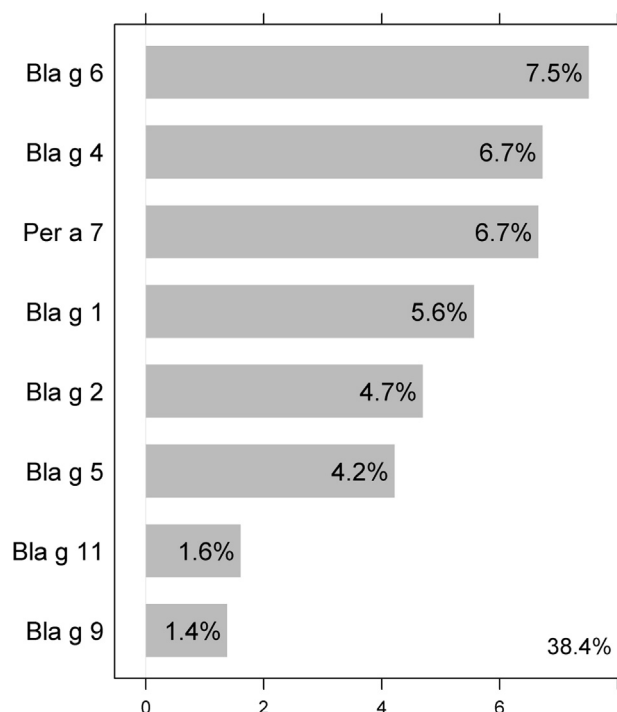


FIG 6. Relative importance for the association of asthma and rhinitis. When combined, these measurements explain 38.4% of the variance. This analysis revealed that Bla g 6, Bla g 4, and Per a 7 were the most important predictors, whereas Bla g 11 and Bla g 9 were the least important in explaining asthma and rhinitis.

prevalence reached 60% to 80% for all allergens except Bla g 1 (30%). Interestingly, differences in IgE prevalence between both groups were greatest for Bla g 1 (7.4-fold), which might indicate that this allergen is an important contributor to the development of disease for patients sensitized to this allergen (26%).

Currently, cockroach extracts are not standardized and have high variability in allergen content.³ Identifying patterns of sensitization to specific cockroach antigens could help guide allergen immunotherapy in efforts to ensure that the patient receives allergens relevant to disease. The ultimate goal is to develop a component diagnostic test to increase the accuracy of the prediction of asthma and rhinitis based on cockroach component analysis. During the ongoing cockroach immunotherapy trials of the Inner-City Asthma Consortium, the cockroach component analysis will allow evaluation of the effectiveness of immunotherapy based on the antigen content of the therapeutic cockroach extract and individual patterns of sensitization.

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Clinical implications: Cockroach-allergic patients with asthma and rhinitis are sensitized at greater levels to more cockroach allergens than patients without disease. Sensitization profiles should be considered for diagnostic and therapeutic purposes.

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