Effects of early-life exposure to allergens and bacteria on recurrent wheeze and atopy in urban children

Susan V. Lynch, PhD,[a]* Robert A. Wood, MD,[b]* Homer Boushey, MD,[a] Leonard B. Bacharier, MD,[c] Gordon R. Bloomberg, MD,[c] Meyer Kattan, MD,[d] George T. O'Connor, MD,[e] Megan T. Sandel, MD,[e] Agustin Calatroni, MS,[f] Elizabeth Matsui, MD,[b] Christine C. Johnson, PhD,[g] Henry Lynn, PhD,[f] Cynthia M. Visness, PhD,[f] Katy F. Jaffee, MS,[f] Peter J. Gergen, MD,[h] Diane R. Gold, MD, MPH,[i] Rosalind J. Wright, MD, MPH,[i] Kei Fujimura, PhD,[a] Marcus Rauch, PhD,[a] William W. Busse, MD,[j] and James E. Gern, MD[j] *San Francisco, Calif, Baltimore, Md, St Louis, Mo, New York, NY, Boston, Mass, Chapel Hill, NC, Detroit, Mich, and Madison, Wis*

Background: Wheezing illnesses cause major morbidity in infants and are frequent precursors to asthma.
Objective: We sought to examine environmental factors associated with recurrent wheezing in inner-city environments.
Methods: The Urban Environment and Childhood Asthma study examined a birth cohort at high risk for asthma (n = 560) in Baltimore, Boston, New York, and St Louis. Environmental assessments included allergen exposure and, in a nested case-control study of 104 children, the bacterial content of house dust collected in the first year of life. Associations were determined among environmental factors, aeroallergen sensitization, and recurrent wheezing at age 3 years.
Results: Cumulative allergen exposure over the first 3 years was associated with allergic sensitization, and sensitization at age 3 years was related to recurrent wheeze. In contrast, first-year exposure to cockroach, mouse, and cat allergens was negatively associated with recurrent wheeze (odds ratio, 0.60, 0.65, and 0.75, respectively; $P \leq .01$). Differences in house dust bacterial content in the first year, especially reduced exposure to specific Firmicutes and Bacteriodetes, was associated with atopy and atopic wheeze. Exposure to high levels of both allergens and this subset of bacteria in the first year of life was most common among children without atopy or wheeze.
Conclusions: In inner-city environments children with the highest exposure to specific allergens and bacteria during their first year were least likely to have recurrent wheeze and allergic sensitization. These findings suggest that concomitant exposure to high levels of certain allergens and bacteria in early life might be beneficial and suggest new preventive strategies for wheezing and allergic diseases. (J Allergy Clin Immunol 2014;■■■:■■■-■■■.)

Key words: *Asthma, atopy, allergen exposure, microbial exposure, inner city*

Wheezing illnesses affect 35% to 50% of children by the age of 3 years[1,2] and are a leading cause for outpatient visits and hospitalizations.[3,4] Wheezing in nonatopic children is often transient, but recurrent wheezing in children with early allergic

From [a]the Department of Medicine, University of California, San Francisco; [b]the Department of Medicine, Johns Hopkins University School of Medicine, Baltimore; [c]the Department of Pediatrics, Washington University School of Medicine and St Louis Children's Hospital, St Louis; [d]the Division of Pediatric Allergy and Immunology, Columbia University Medical Center, New York; [e]the Pulmonary Center, Boston University School of Medicine; [f]Rho, Inc, Chapel Hill; [g]the Division of Allergy and Immunology Henry Ford Health System, Detroit; [h]the National Institute of Allergy and Infectious Diseases, Bethesda; [i]the Channing Laboratory, Department of Medicine, Brigham and Women's Hospital, Harvard Medical School, Boston; and [j]the Department of Pediatrics, University of Wisconsin School of Medicine and Public Health, Madison.

*These authors contributed equally to this work.

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Corresponding author: Robert A. Wood, MD, Johns Hopkins University School of Medicine, 600 North Wolfe St, CMSC 1102, Baltimore, MD 21287. E-mail: rwood@jhmi.edu.

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Abbreviation used
URECA: Urban Environment and Childhood Asthma

sensitization or other signs of atopy during the preschool years is a risk factor for asthma.[5] Because the prevalence and severity of asthma are high in inner cities in the United States, it is especially important to identify risk factors that contribute to the development of allergic sensitization and wheezing in this environment.

The indoor environment of poor urban neighborhoods can include adverse conditions that promote allergic sensitization and recurrent wheezing.[6] Examples include stress, lack of biodiversity, and exposure to indoor pollutants and perennial allergens, such as cockroach and mouse.[7-10] Conversely, farm-related microbial exposures in early life have been linked to protection against allergic diseases.[11,12] Whether relationships exist between microbial exposure and allergic disease outcomes in urban settings is unknown.

To examine the relationship between these conditions and development of allergic sensitization and recurrent wheezing, we studied children enrolled in an ongoing birth cohort study (Urban Environment and Childhood Asthma [URECA]). Per study design, the entire cohort was evaluated at age 3 years to test the hypothesis that high levels of exposure to sensitizing allergens, especially those associated with cockroach and mouse, is associated with the development of allergic sensitization and recurrent wheezing. In addition, a nested case-control study was designed to determine whether early-life exposure to certain microbes in house dust obtained from inner-city homes is associated with development of allergic sensitization and wheezing. The results of these 2 studies are presented in this report.

METHODS

Study design

URECA is a longitudinal birth cohort study in 4 urban areas: Baltimore, Boston, New York City, and St Louis.[13] Selection criteria included residence in an area with more than 20% of residents below the poverty level; mother or father with allergic rhinitis, eczema, and/or asthma; and birth at 34 weeks' gestation or later. Maternal questionnaires were administered prenatally, and participant questionnaires were administered every 3 months thereafter. Clinic visits occurred at 12, 24, 33, and 36 months, and homes were visited annually beginning at age 3 months for an environmental survey and house dust collection. Between February 2005 and March 2007, 1850 families were screened; 889 met the eligibility criteria, and 560 were enrolled. Informed consent was obtained from the parent or legal guardian of the infant.

Study assessments

Allergen-specific IgE (ImmunoCAP; Phadia, Uppsala, Sweden) levels were measured annually for milk, egg, peanut, and German cockroach. At 2 and 3 years of age, specific IgE levels for dust mites, dog, cat, mouse, and *Alternaria* species were also measured. Skin prick testing was performed at age 33 months for 14 common indoor and outdoor allergens.[13]

Household dust samples from the living room (chair or sofa and floor) and child's bedroom (mattress and floor) were collected, as described in the Methods section in this article's Online Repository at www.jacionline.org, and assayed for allergenic proteins, including Bla g 1 (cockroach), Can f 1 (dog), Fel d 1 (cat), Der f 1 and Der p 1 (house dust mites), and Mus m 1 (mouse), by using ELISA (Indoor Biotechnologies, Charlottesville, Va). A subsample (n = 104) of living room dust specimens collected at 3 months of age underwent culture-independent microbiome profiling with a 16S rRNA-based phylogenetic microarray (G3 PhyloChip; Second Genome, San Bruno, Calif; see the Methods section in this article's Online Repository for details) to generate a high-resolution profile of both dominant and rare microbiota members in each sample for comparative and correlative analyses. An approximately equal number of dust samples was randomly selected from each of 4 categories defined by clinical outcomes at age 3 years: (1) recurrent wheeze and aeroallergen sensitivity, (2) recurrent wheeze alone, (3) aeroallergen sensitivity alone, and (4) neither outcome (see Table E1 in this article's Online Repository at www.jacionline.org). This substudy population did not differ from the remainder of the cohort with respect to demographic characteristics or environmental exposures in the first year (see Table E2 in this article's Online Repository at www.jacionline.org).

Definitions

Aeroallergen sensitization was defined by a wheal 3 mm or more larger than that elicited by the saline control on skin prick testing or a specific IgE level of 0.35 kU/L or greater. Recurrent wheeze was defined as parental report of at least 2 wheezing episodes, with at least 1 episode occurring in the third year. Eczema was defined as a score of 1.0 or greater on the Eczema Area and Severity Index[14] at age 3 years. Children at higher risk for asthma were identified by using the modified Asthma Predictive Index.[15]

Statistical analysis

Demographic comparisons between recurrent wheezers and nonwheezers were tested by using Wilcoxon tests for continuous data and χ^2 tests for binary data. Univariate and multivariate analyses to determine association of exposures with sensitivity and recurrent wheeze were performed by using logistic regression. On the basis of this and previous analyses,[16] multivariate models were adjusted for race/ethnicity (strongly correlated with site), sex, mean perceived stress of the mother in the year after birth,[17] and number of smokers in the home.

The 3 allergen exposures showing a strong inverse relationship to recurrent wheeze (cockroach, mouse, and cat; see below) were combined into a single allergen exposure index based on tertiles of exposure to individual allergens (see the Methods section in this article's Online Repository). In addition, a dichotomous variable was created for exposure to each allergen (cockroach, mouse, and cat) to indicate whether the levels were greater than standard cutoffs (Bla g 1, 2 U/g; Mus m 1, 0.5 μg/g; and Fel d 1, 2 μg/g).[18]

Methods used to filter and analyze microbiome data are described in the Methods section in this article's Online Repository.

RESULTS

Factors related to recurrent wheeze in urban children

Of the 560 children in the URECA cohort, 478 (86%) remained in the study at age 3 years; 467 (83%) had sufficient data to assess recurrent wheeze, and 383 (68%) had serum IgE data available at age 3 years. Children included in the primary analysis (with complete follow-up data on wheeze, sensitization, and home allergen exposure data) differed from those not included in terms of study site and race/ethnicity but not allergen exposure (see Table E3 in this article's Online Repository at www.jacionline.org). Of these, 44% were sensitized to at least 1 aeroallergen, 36% had recurrent wheeze, and 9% had eczema (see Fig E1 in this article's Online Repository at www.jacionline.org). Furthermore, 12% of the cohort met the criteria for the modified Asthma Predictive Index, indicating a high risk for subsequent asthma. Factors related to recurrent wheeze were an annual family income of less than $15,000, lower birth weight and gestational age, and the number of smokers in the household

TABLE I. Characteristics of study participants

	Overall (n = 467)	Recurrent wheeze (36%, n = 166)	No recurrent wheeze (64%, n = 301)
Mother completed high school	58% (273)	55% (92)	61% (181)
Mother married	14% (64)	13% (21)	14% (43)
Mother has ever had asthma	50% (234)	55% (91)	48% (143)
Household income <$15,000*	69% (321)	75% (124)	65% (197)
No. of smokers in home*	0.93 ± 1.0	1.1 ± 1.2	0.84 ± 0.91
Child's race/ethnicity			
Black	71% (333)	72% (120)	71% (213)
Hispanic	20% (92)	16% (27)	21% (65)
Other	9% (42)	12% (19)	8% (23)
Child's sex, male	51% (240)	56% (93)	49% (147)
Caesarian section delivery	33% (154)	36% (60)	31% (94)
Child's birth weight (g)*	3258.0 ± 504.4	3203.8 ± 567.0	3287.9 ± 464.6
Gestational age (wk)*	38.8 ± 1.5	38.4 ± 1.6	39.0 ± 1.4
Breast-fed child at 3 mo	24% (100)	20% (29)	26% (71)
BMI (kg/m^2) at age 3	16.7 ± 2.4	16.9 ± 2.9	16.7 ± 2.1

*Significant difference ($P < .05$) between children with recurrent wheeze and children without recurrent wheeze.

TABLE II. Association between allergic sensitization in year 3 with recurrent wheeze in year 3

		Sensitized	Unadjusted		Adjusted (+ race and sex)		Adjusted (+ race, sex, PSS, and ETS)	
Sensitizations	n	Percent (no.)	Odds ratio (95% CI)	*P* value	Odds ratio (95% CI)	*P* value	Odds ratio (95% CI)	*P* value
Any food	383	40% (155)	1.74 (1.14-2.67)	.01	1.81 (1.18-2.79)	.007	1.94 (1.25-3.03)	.003
Any aeroallergen	356	46% (163)	1.58 (1.02-2.46)	.04	1.53 (0.98-2.39)	.06	1.63 (1.03-2.56)	.04
Cat	362	17% (61)	1.78 (1.02-3.12)	.04	1.80 (1.03-3.17)	.04	1.93 (1.09-3.44)	.03
Dog	359	12% (44)	1.91 (1.01-3.60)	.05	1.86 (0.97-3.55)	.06	2.22 (1.14-4.34)	.02
Cockroach	359	14% (52)	1.62 (0.89-2.94)	.11	1.58 (0.87-2.89)	.14	1.74 (0.94-3.21)	.08
Mouse	366	20% (74)	1.58 (0.94-2.66)	.09	1.60 (0.95-2.72)	.08	1.74 (1.01-2.97)	.04
Dust mite *(Dermatophagoides farinae)*	364	11% (41)	2.18 (1.13-4.20)	.02	2.08 (1.07-4.03)	.03	2.40 (1.22-4.72)	.01
Dust mite *(Dermatophagoides pteronyssinus)*	360	13% (45)	1.48 (0.79-2.80)	.23	1.44 (0.76-2.73)	.27	1.57 (0.82-3.03)	.17

ETS, Environmental tobacco smoke; *PSS*, Perceived Stress Scale.

(Table I). Children with recurrent wheeze had a median of 6 wheezing episodes (range, 2-26 wheezing episodes) by age 3 years, and 77% had been prescribed albuterol, 27% had been prescribed an inhaled corticosteroid, and 33% had been prescribed at least 1 course of oral corticosteroid for wheezing.

Allergen levels from the first-year dust samples varied by site (see Fig E2 in this article's Online Repository at www.jacionline.org), and there was also moderate within-subject variation over the 3 years (intraclass correlation, 0.32-0.56). Cumulative exposure (summed over the 3 years) to cockroach, mouse, and dust mite *(Dermatophagoides farinae)* was positively associated with sensitization to those allergens at age 3 years (odds ratio, 1.27-1.68; see Table E4 in this article's Online Repository at www.jacionline.org), and as expected, allergic sensitization was positively associated with recurrent wheeze (Table II). In contrast, exposure to allergens in the first year had little or no association with sensitization at age 3 years (see Table E4).

However, in contrast to our expectations, significant inverse relationships were found between first-year exposure to cockroach, mouse, and cat, but not house dust mite or dog, allergens and recurrent wheeze at age 3 years (odds ratio, 0.60, 0.65, 0.75, 0.97, and 1.01, respectively; Table III). An additive reduction in wheeze was observed with exposure to more than 1 of these 3 allergens (Fig 1). When categorized as exposed or not exposed by using standard cutoffs, recurrent wheeze decreased from 51% in those with no exposures (n = 96) to 17% in those exposed to all 3 allergens (n = 18; Fig 1, *A*). By using an index that reflects exposure to all 3 of these allergens in the first year of life, the prevalence of recurrent wheeze was inversely related to the index over the entire range of exposures (Fig 1, *B*). The negative relationship between first-year exposures and wheeze persisted when stratified by aeroallergen sensitization and when adjusted for covariates (Fig 1, *C*, and Table III). Our data revealed that the timing of allergen exposure was important; the inverse relationship with recurrent wheeze at age 3 years was significant only for exposures in the first year of life and not for those encountered in the second or third years (Fig 1, *D* and *E*).

Relationship of microbial exposure to atopy and recurrent wheeze

To test the hypothesis that early-life exposure to certain microbes in house dust is associated with protection against allergic sensitization and wheezing, we conducted a nested case-control study of the microbes present in house dust of 104 URECA households. The number of samples were relatively evenly distributed across 4 distinct groups: children with recurrent wheeze alone ("Wheeze," n = 26), atopy alone ("Atopy," n = 25), both recurrent wheeze and atopy

TABLE III. Association between bedroom dust allergen exposure in the first year of life with recurrent wheeze in year 3

		Unadjusted		Adjusted (+ sensitization*)		Adjusted (+ race and sex)		Adjusted (+ race, sex, PSS, and ETS)	
	No.	Odds ratio (95% CI)	*P* value	Odds ratio (95% CI)	*P* value	Odds ratio (95% CI)	*P* value	Odds ratio (95% CI)	*P* value
Exposures (year 1)†									
Cat	362	0.75 (0.61-0.92)	.005	0.74 (0.61-0.91)	.004	0.73 (0.60-0.90)	.003	0.71 (0.58-0.88)	.001
Dog	359	1.01 (0.80-1.27)	.97	1.01 (0.80-1.27)	.97	1.04 (0.82-1.32)	.76	1.00 (0.79-1.28)	.98
Cockroach	359	0.60 (0.45-0.80)	<.001	0.59 (0.44-0.79)	<.001	0.61 (0.45-0.81)	<.001	0.59 (0.44-0.80)	<.001
Mouse	366	0.65 (0.52-0.82)	<.001	0.64 (0.50-0.81)	<.001	0.64 (0.51-0.81)	<.001	0.65 (0.51-0.82)	<.001
Dust mite *(Dermatophagoides farinae)*	364	0.97 (0.78-1.20)	.78	0.96 (0.77-1.19)	.69	0.95 (0.76-1.18)	.63	0.92 (0.73-1.15)	.45
Model using allergen exposure index‡									
Exposure index (0-9)	356	0.73 (0.64-0.83)	<.001	0.73 (0.64-0.83)	<.001	0.73 (0.64-0.84)	<.001	0.73 (0.64-0.83)	<.001
Allergen exposure index stratified by aeroallergen sensitivity‡									
Not sensitive	234	0.73 (0.61-0.88)	0.95§	NA	NA	0.73 (0.60-0.88)	0.97§	0.72 (0.60-0.88)	.91§
Sensitive	122	0.73 (0.61-0.88)		NA	NA	0.74 (0.61-0.89)		0.73 (0.61-0.89)	

ETS, Environmental tobacco smoke; *NA*, not applicable; *PSS*, Perceived Stress Scale.
*Individual exposure models are adjusted for the respective sensitization. The exposure index is adjusted for overall aeroallergen sensitization.
†Odds of recurrent wheeze for each 1-log increase in allergen level.
‡Odds of recurrent wheeze for a 1-unit increase in the allergen exposure index.
§Interaction *P* value.

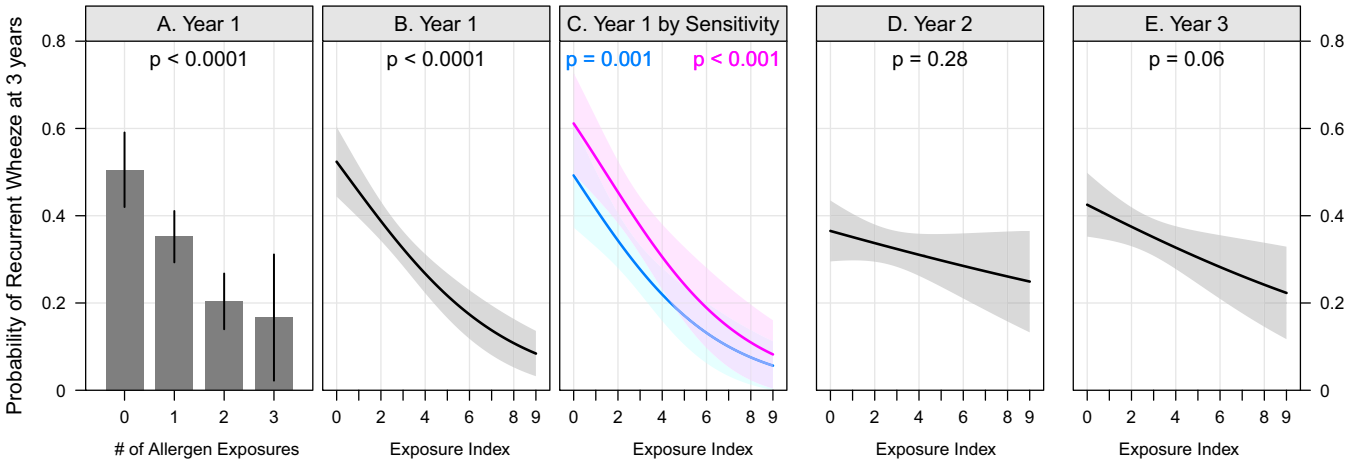

FIG 1. Relationships between specific allergen exposures and recurrent wheezing. **A,** Probability of recurrent wheeze (95% CIs) according to the number of allergen exposures (cockroach, mouse, and/or cat). **B-E,** Probability of recurrent wheeze (95% CI) determined by using logistic regression. Fig 1, *C* is shown stratified by aeroallergen sensitivity (*pink* = sensitive, *blue* = not sensitive). Fig 1, *D* and *E*, show the probability of recurrent wheeze for allergen exposures during years 2 and 3.

("Both," n = 24), or neither recurrent wheeze nor atopy ("Neither," n = 29). The inverse association between first-year allergen exposure and recurrent wheeze at age 3 years in the nested case-control study population was similar to that observed in the whole population (see Table E5 in this article's Online Repository at www.jacionline.org).

Relative bacterial richness, a measure of the number of bacterial taxa detected in each sample, was significantly different among the 4 groups ($P < .02$) and was considerably lower in first-year dust samples from the Atopy or Both groups relative to the Neither group (see Fig E3 in this article's Online Repository at www.jacionline.org), indicating reduced bacterial exposure in these environments. Multivariate analysis of house dust bacterial community composition further demonstrated that the Neither group house dust microbiome was compositionally distinct from that of the Atopy ($P = .002$; Fig 2, *A*) and Both ($P = .005$; Fig 2, *B*) groups but not from the Wheeze group (Fig 2, *C*).

To identify the specific taxa within the house dust microbiome that discriminated among these groups, we next compared taxon relative abundance between the Neither group and each of the other groups. The greatest number of taxa exhibiting significant difference in relative abundance was identified in the Both versus Neither comparison (82 taxa; Fig 2, *E*). All of these "taxa of interest" were significantly increased in the first-year dust samples of the Neither group, raising the possibility that exposure to certain bacteria in house dust during the first year of life might play a role in protection against atopic wheeze. These putatively protective bacteria belonged to several phyla but were primarily members of the Bacteroidetes and Firmicutes, particularly the Prevotellaceae, Lachnospiraceae, and Ruminococcaceae families (see Table E6 in this article's Online Repository at

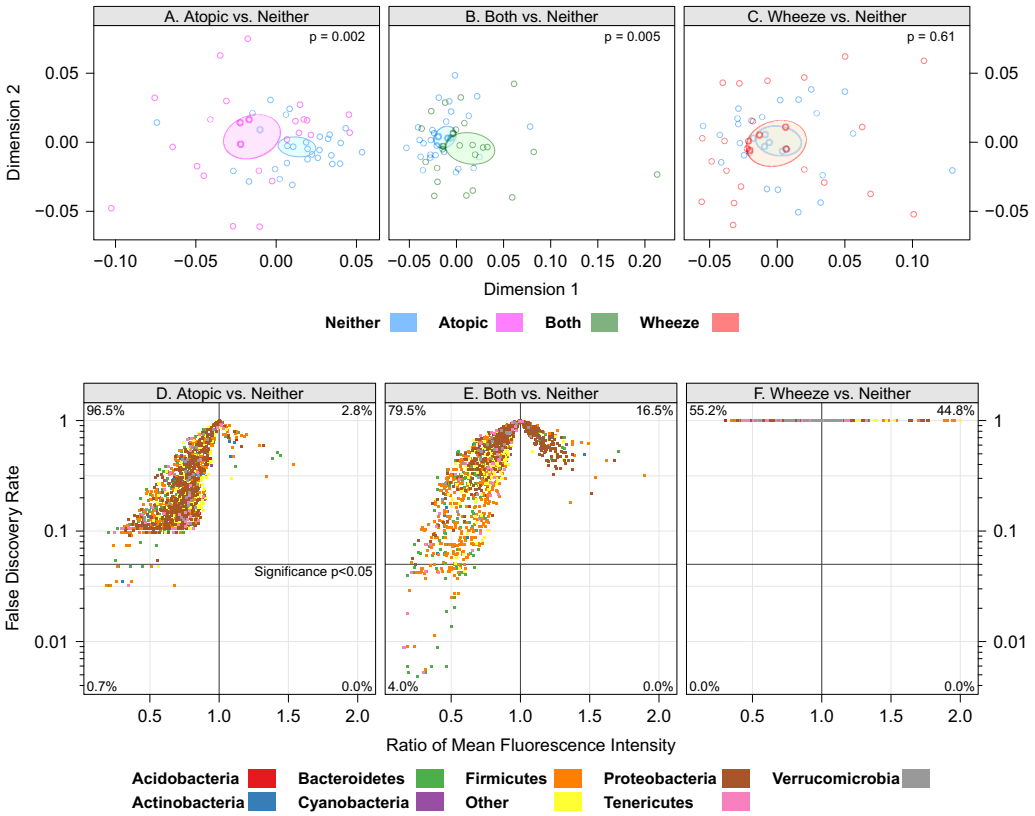

FIG 2. House dust microbiome composition is associated with clinical outcomes. **A-C,** Similarity or dissimilarity of house dust microbiota composition is indicated by the distance between samples *(colored dots)*. Samples plotted close together are compositionally similar, and greater intersample distance indicates compositionally distinct bacterial communities. *Ellipses* represent 95% CIs for each group. **D-F,** Mean taxon relative abundance across house dust samples from the Neither group was compared with those of the Atopy (Fig 2, *D*), Both (Fig 2, *E*), and Wheeze (Fig 2, *F*) groups to identify specific bacterial taxa associated with clinical outcomes. Taxa in the left lower quadrant are underrepresented in the Atopy (Fig 2, *D*) and Both (Fig 2, *E*) groups compared with the Neither group. Colors indicate phylum-level classification of individual taxa, and the *horizontal lines* indicate *P* values of less than .05 after correction for false discovery rates.

www.jacionline.org). Fewer taxa showed significant differences in relative abundance in the Neither versus Atopy group comparison (Fig 2, *D*), but again, of those that did, all were relatively more abundant in the Neither group and were thus associated with a reduction in the risk of atopy. These taxa largely belonged to the same families as those identified from the Neither versus Both group comparison (see Table E6). No taxa were found to differ significantly in relative abundance between the Neither and Wheeze groups (Fig 2, *F*).

Relationships between allergen and microbial exposures

We next asked whether bacteria that were inversely related to atopy and atopy with wheeze were also related to allergen exposure. Of the 82 taxa of interest significantly enriched in the Neither group, 14 and 10 were also significantly correlated with levels of Mus m 1 and Bla g 1 allergens, respectively, and these taxa were primarily members of the Bacteroidetes (eg, Prevotellaceae and Rikenellaceae; see Table E7 in this article's Online Repository at www.jacionline.org). *Blattabacterium* species, known endosymbionts of cockroaches,[19] were highly positively correlated with Bla g 1 levels, indicating that some of these bacterial constituents of house dust might originate from the enteric contents of cockroaches. None of the 82 taxa of interest were associated with Fel d 1 allergen levels.

Relationship of combined effects of allergens and bacteria to study outcomes

Given the above associations, we thought it important to examine how combined early-life exposures to the selected allergens (cockroach, mouse, and cat) and bacteria (richness of either the total community or the 82 taxa of interest) affected clinical outcomes at 3 years. Our analysis of the nested case-control study population showed that the combined exposure patterns differed significantly across the 4 outcome groups, and this was true whether the richness of the total bacterial community (Fig 3, *A*) or of the 82 taxa of interest (Fig 3, *B*) was considered in the analysis. Of the children in the Both group, the largest proportion (42%) were exposed to low levels of allergens and low bacterial community richness, whereas the smallest proportion (8%) of this group had been exposed to high levels of selected allergens and bacterial richness. Conversely, the children in the Neither group were more likely to have been exposed to high levels of allergens and high bacterial richness (41%) and

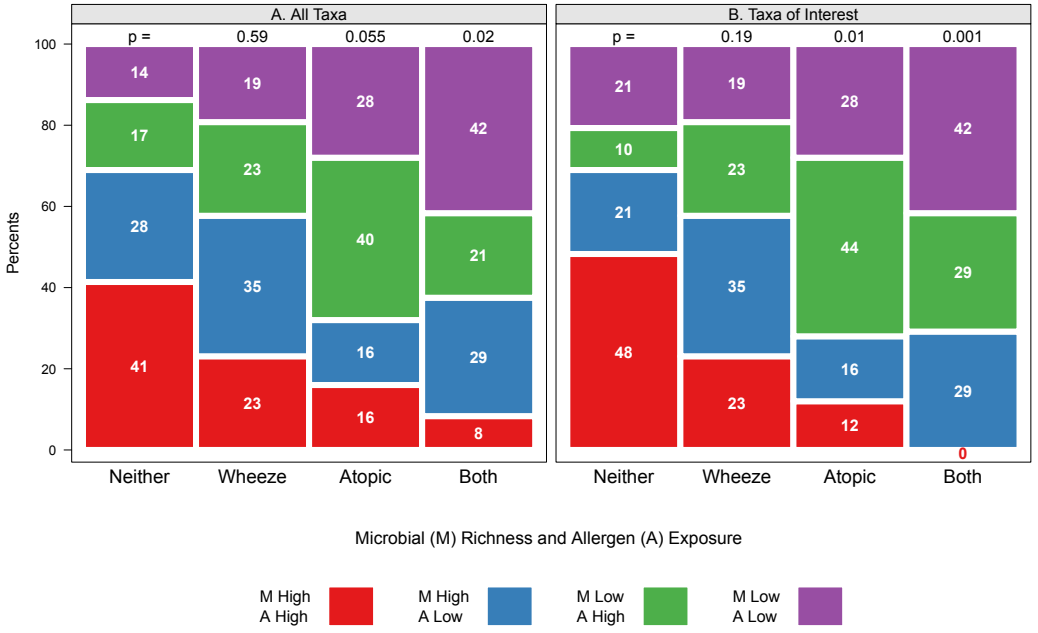

FIG 3. Distribution of allergen and bacterial exposure among children with atopy, recurrent wheeze, atopy with wheeze, and neither outcome. Exposure to allergen is classified as high or low with respect to the median of the allergen exposure index and richness of exposure to all microbes **(A)** or taxa of interest **(B)**. The distribution of exposure in each outcome group is compared with the distribution in the Neither group.

were least likely (14%) to have had low-level allergen and bacterial exposure (Fig 3). These comparisons reached higher levels of significance when only the 82 taxa of interest were used in the analysis (Fig 3, *B*). For example, the largest proportion (42%) of children in the Both group had low-level exposure to both the specified allergens and the 82 bacteria of interest, whereas none of the children in this group were exposed to high levels of allergen and of these specific bacteria.

DISCUSSION

Using a birth cohort and a nested case-control study, we assessed the relationships between exposure to allergens and bacteria over the first 3 years of life and the development of recurrent wheeze and atopy. As hypothesized, cumulative allergen exposure over the first 3 years of life was associated with allergic sensitization, and allergic sensitization was associated with recurrent wheeze. However, the major finding from the birth cohort study was that high levels of cockroach, mouse, and, to a lesser extent, cat allergen in the first-year inner-city house dust samples had a strong inverse relationship with recurrent wheeze at age 3 years. This association was strongest for allergen levels in the first-year dust samples, suggesting that the first few months of life is a critical time period in childhood allergic disease development. In addition, changes in the bacterial house dust environment, characterized by reduced exposure to bacterial richness, as well as specific bacteria, were significantly associated with the development of atopy with wheezing or atopy alone. Some of the bacterial taxa related to favorable clinical outcomes were positively associated with mouse and cockroach allergen levels, raising the possibility that household pests might be the source of some of the beneficial bacteria in the inner-city environment. Finally, combined analysis of exposure to both allergens and bacteria revealed that the group of children with neither wheeze nor atopy had the highest first-year exposure to allergens and the bacterial species identified in this study as potentially protective against atopy and recurrent wheeze. To our knowledge, this is the first scientific report of exposure to high levels of allergens combined with an environment rich in specific bacterial families as having a protective effect against atopy and atopy with wheezing in early childhood.

A prevalent conceptual model is that exposure to perennial indoor allergens contributes to the development of allergic sensitization with subsequent development of wheezing.[20-22] Earlier cross-sectional studies of inner-city populations supported this concept by showing a dose-response relationship between Bla g 1 levels in house dust and allergic sensitization to cockroach.[23] Our findings are in some ways consistent with this model in showing that cumulative exposure to cockroach and mouse allergens in the dust of inner-city houses over the first 3 years of life is associated with allergic sensitization and that allergic sensitization is associated with recurrent wheeze.

Where our findings importantly differ from this conceptual model is in showing an inverse relationship between the levels of cockroach, mouse, and cat allergen in dust samples collected in the first months of life and recurrent wheeze at age 3 years. The temporal nature of the relationship is similar to that previously observed between early-life exposure to pets and reduced rates of allergic sensitization and asthma[24-26] and supports a mounting body of evidence that exposures in the first few months of life are important in shaping allergic and respiratory outcomes. This counterintuitive effect of early-life exposure to certain allergens might help explain the failure of several previous studies to find straightforward relationships between allergen exposure and the development of atopy and asthma[27-30] or to demonstrate that

allergen avoidance protects against development of these conditions.[31-34] Notably, in contrast to other reports,[25,35] dog exposure was not protective for atopy or wheeze in this study. We speculate that the reason for the lack of protection in urban homes might be that dogs can be kept for security and thus interact less with young children and might also be less likely to bring soil into the home from outdoor sources, which are sparse in urban neighborhoods.

Our findings point to particular families of bacteria as being protective against the development of atopy and atopic wheeze. These findings are in line with experimental data in animals and observational studies in Western Europe showing that microbial exposures in early life are critical contributors to respiratory health.[11,19] Certainly, the URECA findings have a very different context; overall rates of wheezing and allergic sensitization in low-income, urban US populations are high, likely influenced by adverse factors related to the urban environment (eg, indoor and outdoor pollutants) and increased rates of low birth weight, prematurity, household tobacco smoking, and maternal stress and depression. Among families exposed to these adverse conditions, it is remarkable that the general tenets of the hygiene hypothesis are applicable, in that some of the residences in this environment contain bacteria that reduce the risk of allergic sensitization with or without recurrent wheezing through exposure. Whether these bacteria are similar in phylogeny, quantity, or function to those found in farmhouses remains to be determined.

The URECA study is the first to consider exposures to both allergens and bacteria in the same population. The effects of high specific allergen and specific bacterial exposure appear to be at least partially independent. High cockroach, mouse, and cat allergen exposure was associated with reduction in recurrent wheezing regardless of atopy, whereas higher exposure to specific bacterial families in house dust appeared to target atopy. Interpretation of these effects is complicated by the fact that some of the "protective" bacteria were positively related to allergen levels. The combined analysis revealed potential synergism between high allergen and bacterial exposures: the children with neither atopy nor wheezing at age 3 years had high early-life exposure to both allergens and the bacterial families of interest. In contrast, children with atopy alone were most often exposed to high levels of allergens and low levels of the putatively protective bacterial families. These clinical findings are consistent with experimental models in which induction of tolerance is achieved through administration of allergen together with a tolerizing signal provided by specific microbes.[36-38] Our observations raise the possibility that the optimal conditions to promote tolerance might be early-life antigenic exposure to allergen in conjunction with exposure to particular bacteria, such as those identified in this study.

There are several possible explanations for the associations between early microbial and allergen exposures and 3-year outcomes. The infant microbiome exhibits a temporal program of community assembly over the first year of life, and patterns of early-life gastrointestinal colonization have previously been linked to immune development,[39] allergic disease,[40,41] and the response to viral respiratory tract infection.[42,43] Most of the "protective" taxa identified in our study belong to bacterial families (eg, Prevotellaceae, Lachnospiraceae, and Ruminococcaceae), which are human colonizers and important producers of immunomodulatory metabolites, such as short-chain fatty acids.[44] This suggests that early-life exposure to house dust containing these bacteria might inoculate the developing gastrointestinal microbiome with species that produce metabolites that protect against development of atopy and atopic wheeze. It is also plausible that these microbial exposures could exert a similar effect at airway mucosal surfaces. Accordingly, the respiratory microbiome is altered in asthmatic patients,[45,46] and experiments in mice demonstrate that environmental microbes in early life can modify lung mucosal immunity to reduce inflammatory responses to allergens.[47] How allergen exposure modifies the risk of recurrent wheeze, which frequently is caused by viral respiratory infections, is unknown, but the mechanisms could be related to direct biological effects of allergens (eg, activation of innate immune receptors) or allergen-associated biologic agents (eg, proteases, chitin, and DNA).[48-50] Whether exposure to these immunologically active substances in early life affects the development of mucosal immune and antiviral responses remains to be determined.

The strengths of our investigation include its examination of an inner-city population at high risk for respiratory morbidity, high rate of study participant retention, and prospective assessments of environmental exposures. Previous clinical studies of the microbiome have used lower-resolution culture- or molecular-based approaches to distinguish a limited selection of microbes. The URECA study used a phylogenetic microarray to profile thousands of organisms in parallel and provided data at the phylum, family, and species level. This is a multicenter study with site differences in exposure, race/ethnicity, and the prevalence of recurrent wheeze, but the main study findings were unaffected by adjustment for these factors. One limitation of the study is that the microbial analysis was performed in a subset of homes, and therefore relationships between specific bacterial taxa and outcomes, although false discovery rate corrected, await confirmation in other populations. Furthermore, the array-based method in this study provides neither information about absolute quantity of bacteria nor functional analysis of metabolic activity that could mediate effects on immune development. Finally, it is important to consider that effects of environmental exposures could change with age and also in relationship to age-dependent phenotypes (eg, recurrent wheezing vs persistent asthma).

In summary, the inner-city environment includes adverse exposures that promote allergic disease and wheezing. Even so, our results demonstrate that within this environment, early-life exposures to certain allergens and bacteria are associated with significant reductions in recurrent wheeze and atopy. Although allergen avoidance can benefit children with established allergic asthma, our findings imply that different strategies will be needed to prevent atopy and wheeze in preschool children. Conceptually, in environments characterized by high allergen exposure, our results raise the possibility that enhancing microbial exposures could be more effective than allergen abatement. Moving forward, the challenge will be to define whether specific allergen- and bacteria-associated mechanisms account for these effects to enable formulation of evidence-based interventions.

The URECA study is a collaboration of the following institutions and investigators (principal investigators are indicated by an asterisk and the protocol chair is indicated by double asterisks): *Johns Hopkins University, Baltimore, Maryland*: R. Wood,* E. Matsui, H. Lederman, F. Witter, J. Logan,

S. Leimenstoll, D. Scott, L. Daniels, L. Miles, D. Sellers, A. Swift; *Boston University School of Medicine, Boston, Massachusetts*: G. O'Connor,* W. Cruikshank, M. Sandel, A. Lee-Parritz, C. Stamoulos, E. Gjerasi, P. Price-Johnson, B. Caldwell, M. Tuzova; *Harvard Medical School, Boston, Massachusetts*: D. Gold, R. Wright; *Columbia University, New York, NY*: M. Kattan,* C. Lamm, N. Whitney, P. Yaniv, C. Sanabia, A. Valones; *Mount Sinai School of Medicine, New York, NY*: H. Sampson, M. Mishoe; *Washington University School of Medicine, St Louis, Missouri*: G. Bloomberg,* L. Bacharier, Y. Sadovsky, E. Tesson, C. Koerkenmeier, R. Sharp, K. Ray, J. Durrange, I. Bauer; *Statistical and Clinical Coordinating Center, Rho, Inc, Chapel Hill, NC*: H. Mitchell,* P. Zook, C. Visness, M. Walter, R. Bailey, S. Hicks, W. Taylor, R. Budrevich; *Scientific Coordination and Administrative Center, University of Wisconsin, Madison, Wisconsin*: W. Busse,* J. Gern,** P. Heinritz, C. Sorkness, W. M. Lee, K. Grindle, A. Dresen, T. Pappas; *National Institute of Allergy and Infectious Diseases, Bethesda, Maryland*: P. Gergen, A. Togias, E. Smartt, K. Thompson.

Clinical implications: Concomitant exposure to high levels of certain allergens and bacteria in early life might be beneficial, which suggests new preventive strategies for wheezing and allergic diseases.

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METHODS

Collection of dust samples

URECA staff collected settled dust samples from children's homes with a Mitest Dust Collector (Indoor Biotechnologies, Charlottesville, Va) and a vacuum cleaner (Oreck Super-Deluxe Compact Canister, Model BB870AD; Oreck, New Orleans, La). At each home visit, 3 dust samples are collected: (1) a combined sample from the child's bed and bedroom floor, (2) an extra sample of dust from the child's bedroom floor, and (3) a combined sample from the family room floor and sofa or chair. For analysis of bedroom exposures, dust samples from the combined bed/floor sample were used primarily, and if the quantity of dust was insufficient, this dust was supplemented with the second sample of dust collected from the bedroom floor. Detailed methods for dust collection and processing are as previously described.[E1]

Allergen exposure index

The 3 allergen exposures showing a strong relationship to recurrent wheeze (cockroach, mouse, and cat; see below) were combined into a single allergen exposure index based on tertiles of exposure to individual allergens. First, each exposure was divided into 4 categories (0 = no allergen detected; 1-3 = tertiles of the allergen level above detection). The categories were then summed, creating a score ranging from 0 to 9.

Microbial analysis

For the nested case-control study, we examined living room dust specimens using a culture-independent microbiome profiling 16S rRNA-based phylogenetic microarray (G3 PhyloChip; Second Genome, San Bruno, Calif) to generate a high-resolution profile of both dominant and rare microbiota members in each sample profiled.[E2] DNA was extracted by using the Hexadecyltrimethylammonium bromide (CTAB) method, and PCR amplification of the 16S rRNA gene for PhyloChip analysis was performed in 25-μL reactions.[E3] The G3 PhyloChip was used to provide a high-resolution profile of the microbial community present and to permit standardized quantification of approximately 60,000 distinct bacterial taxa. Because it consists of oligonucleotides of distinct sequence with different hybridization efficiencies, this tool cannot provide relative distribution of taxa within a single sample. In addition, because it is hybridization based, some taxa might be identified because of cross-hybridization, although this is minimized through the use of stringent data-filtering criteria. We have previously demonstrated the validity of this tool and its utility in detecting significantly greater numbers of bacterial taxa within communities compared with next-generation sequencing platforms using typical read depth (ie, approximately 30,000 reads per sample).[E2] Moreover, using this array as a relatively inexpensive high-resolution microbiome screening tool, we have successfully identified species associated with particular chronic inflammatory diseases, such as chronic rhinosinusitus,[E4] or, more recently, the specific subcommunity of organisms highly correlated with particular immune phenotypes associated with HIV disease progression.[E5] A total of 500 ng of purified PCR product spiked with 96 non-16S rRNA quantitative standards per sample was fragmented, biotin labeled, and hybridized to the G3 16S rRNA PhyloChip, as previously described.[E6] Fluorescent images were captured with the GeneChip Scanner 3000 7G (Affymetrix, Santa Clara, Calif). Probe intensities were background subtracted and scaled to the quantitative standards, and outliers were identified.[E7] Data were filtered with the empiric Operational Taxonomic Unit approach (DeSantis, personal communication). Data were natural log transformed before subsequent analyses. From each house dust sample, mean fluorescence intensities (reflective of relative abundance) of all taxa (groups of bacteria ≥99% 16S rRNA sequence homology) passing these data-filtering criteria (approximately 2000 taxa) were subject to analyses.

Statistical methods

Nonmetric multidimensional scaling with a Canberra distance matrix and the Vegan package in the R statistical package (http://www.r-project.org) was used to examine pairwise compositional dissimilarity across house dust microbiota profiles, and permutational ANOVA was performed in the Adonis package (also in R) to determine how clinical outcomes were associated with observed variation in community composition. Between-group comparative analysis of taxon relative abundance was performed by using a moderated *t* test with Limma in R, with results ranked by the strength of the Benjamini-Hochberg false discovery rate–corrected *P* value. Correlations were summarized by using Pearson correlations, and results were also subject to false discovery correction. Figures were programmed in R version 3.0.2.

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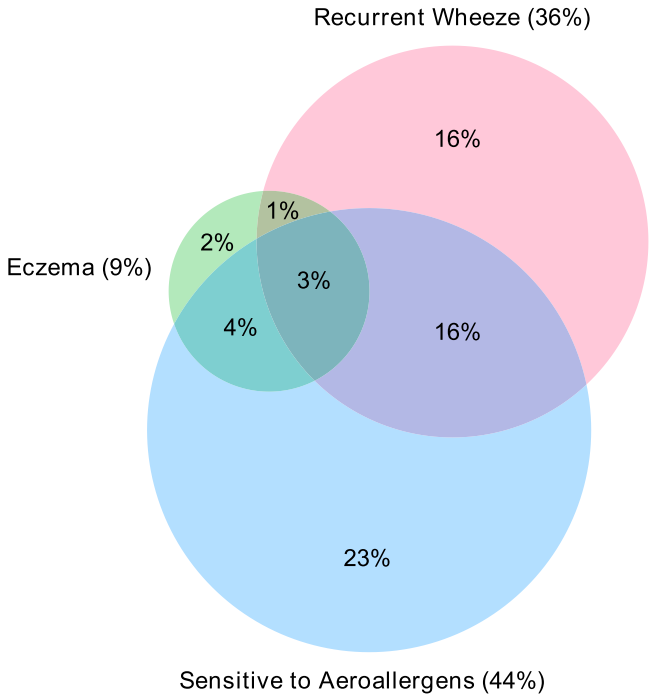

FIG E1. Relationship of recurrent wheeze, aeroallergen sensitization, and eczema at age 3 years. The Venn diagram illustrates overall rates and interrelationships between recurrent wheeze, sensitization to at least 1 aeroallergen, and eczema.

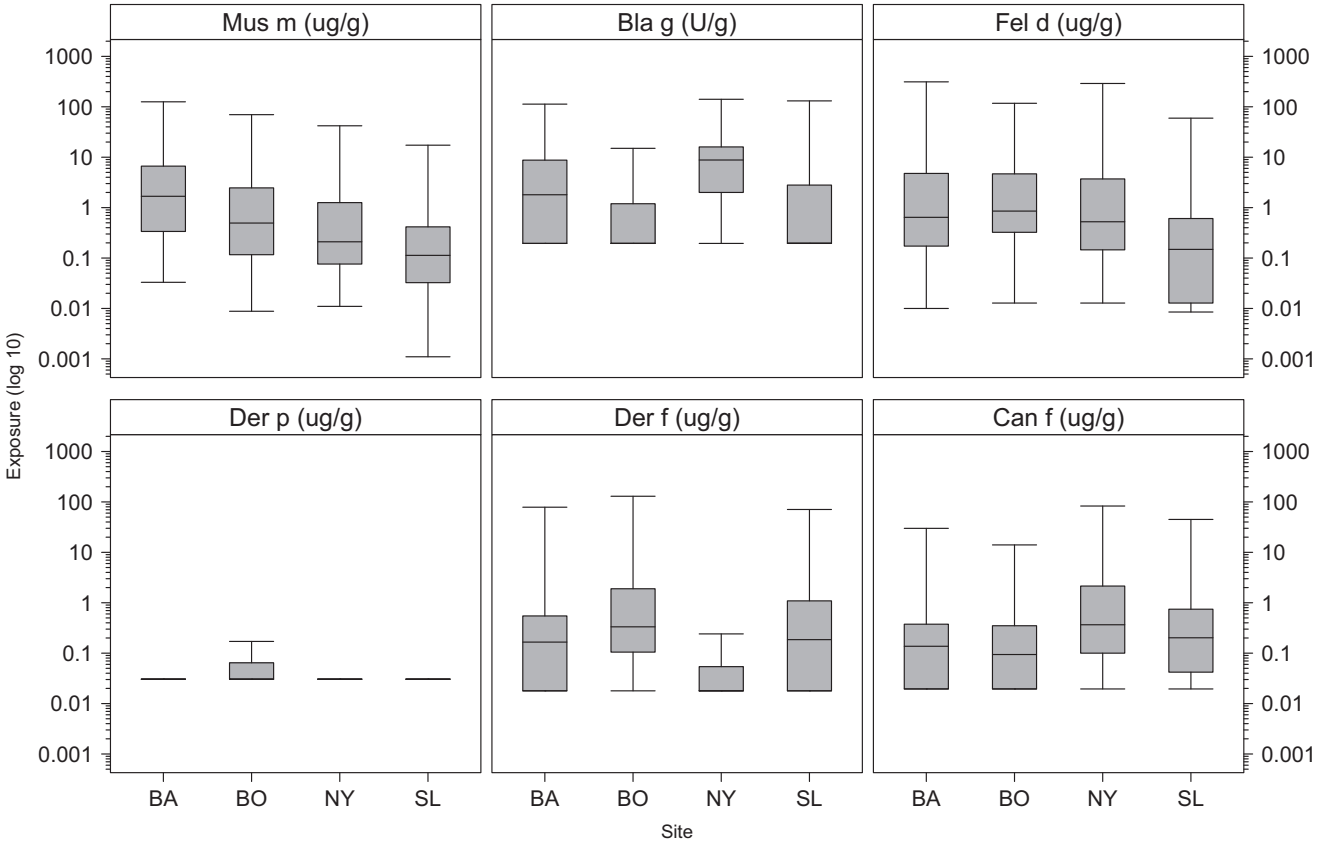

FIG E2. Distribution of first-year bedroom allergen levels by site. The *box plots* represent 25th and 50th percentiles, medians are represented by *horizontal lines*, and *whisker bars* represent the 10th and 90th percentiles. *BA*, Baltimore; *BO*, Boston; *NY*, New York; *SL*, St Louis.

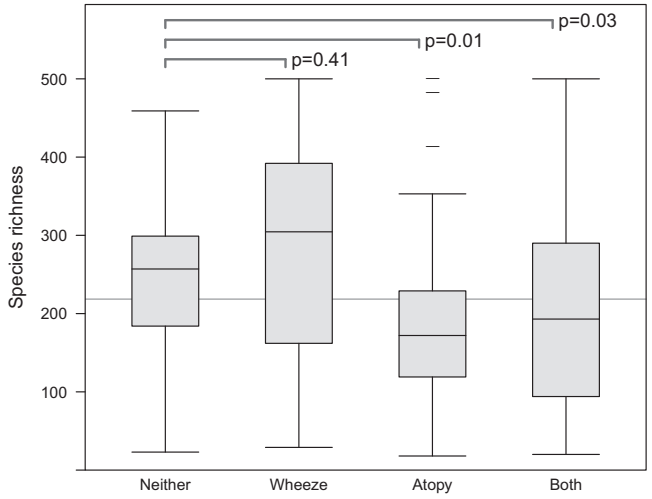

FIG E3. Median bacterial taxa richness by study group in the nested case-control study. *P* values represent pairwise comparisons with the Neither group, and the *horizontal line* illustrates the overall median value for bacterial richness for all groups combined. Overall $P = .02$.

TABLE E1. Prevalence of recurrent wheeze and atopy at 3 years in the microbiota and URECA populations

	Microbiota population (n = 104)*	3-y Population (n = 383)*	3-y Population (n = 418)†
Recurrent wheeze only	25% (26/104)	22% (86/383)	17% (73/418)
Atopy only	24% (25/104)	15% (56/383)	26% (108/418)
Neither	28% (29/104)	50% (193/383)	38% (160/418)
Both	23% (24/104)	13% (48/383)	18% (77/418)

*The definition of atopy is an IgE level of 0.35 kUa/L or greater.
†The definition of atopy is an IgE level of 0.35 kUa/L or greater or positive skin test result (wheal ≥ 3 mm).

TABLE E2. Characteristics of the selected microbiota sample and the remainder of the URECA study population

	Microbiota sample (n = 104)	Remainder of URECA population (n = 505)	*P* value
Site			.17
Baltimore	28% (29)	27% (136)	
Boston	24% (25)	24% (121)	
New York	12% (13)	21% (107)	
St Louis	36% (37)	28% (141)	
Child's race/ethnicity			.39
Black	76% (79)	69% (347)	
Hispanic	16% (17)	22% (108)	
Other	8% (8)	10% (46)	
Mother married	13% (14)	13% (66)	.99
Mother completed high school	59% (61)	59% (295)	.99
Household income <$15,000	72% (75)	68% (327)	.46
Gestational age (wk)	38.7 ± 1.4	38.7 ± 1.5	.98
Child's birth weight (g)	3244.4 ± 470.3	3232.9 ± 522.0	.82
Breast-fed child at 3 mo	21% (22)	25% (101)	.47
No. of smokers in home	0.97 ± 0.95	0.88 ± 1.02	.37
Der p 1, bedroom (μg/g)	0.44 (0.44-0.44)	0.44 (0.44-0.44)	.86
Der f 1, bedroom (μg/g)	0.28 (0.26-0.33)	0.27 (0.26-0.34)	.41
Fel d 1, bedroom (μg/g)	0.31 (0.20-1.04)	0.32 (0.20-0.79)	.84
Can f 1, bedroom (μg/g)	0.00 (0.00-0.04)	0.00 (0.00-0.01)	.68
Bla g 1, bedroom (U/g)	0.60 (0.20-7.15)	1.09 (0.20-6.47)	.94
Mus m 1, bedroom (μg/g)	0.49 (0.10-2.95)	0.39 (0.10-2.53)	.97
Der p 1, living room (μg/g)	0.44 (0.44-0.44)	0.44 (0.44-0.44)	.73
Der f 1, living room (μg/g)	0.27 (0.26-0.30)	0.27 (0.26-0.30)	.83
Fel d 1, living room (μg/g)	0.38 (0.21-1.52)	0.37 (0.23-1.77)	.67
Can f 1, living room (μg/g)	0.00 (0.00-0.06)	0.00 (0.00-0.10)	.35
Bla g 1, living room (U/g)	0.65 (0.20-5.35)	1.07 (0.20-7.32)	.39
Mus m 1, living room (μg/g)	0.42 (0.08-2.12)	0.64 (0.12-3.50)	.17

Values are presented as medians (interquartile ranges), mean ± standard deviation, or percentages (numbers).

TABLE E3. Characteristics of participants included and not included in analyses of 3-year outcomes

	Included in analysis population (n = 359)	Not included in analysis population (n = 201)	*P* value
Site			<.01
Baltimore	30% (109)	24% (49)	
Boston	25% (89)	21% (43)	
New York	15% (55)	31% (60)	
St Louis	30% (106)	24% (49)	
Child's race/ethnicity			.02
Black	74% (264)	64% (125)	
Hispanic	17% (62)	27% (53)	
Other	9% (33)	9% (19)	
Mother married	15% (53)	10% (19)	.13
Mother completed high school	61% (218)	53% (103)	.10
Household income <$15,000	69% (249)	67% (120)	.65
Gestational age (wk)	38.8 ± 1.5	38.6 ± 1.6	.18
Child's birth weight (g)	3249.7 ± 497.8	3213.8 ± 543.3	.44
Breast-fed child at 3 mo	23% (79)	24% (31)	.92
No. of smokers in home	0.92 ± 1.04	0.88 ± 0.97	.66
Der p 1, bedroom (μg/g)	0.44 (0.44-0.44)	0.44 (0.44-0.44)	.92
Der f 1, bedroom (μg/g)	0.27 (0.26-0.33)	0.28 (0.26-0.32)	.21
Fel d 1, bedroom (μg/g)	0.34 (0.20-1.20)	0.30 (0.20-0.70)	.37
Can f 1, bedroom (μg/g)	0.00 (0.00-0.03)	0.00 (0.00-0.04)	.33
Bla g 1, bedroom (U/g)	0.93 (0.20-6.30)	0.41 (0.20-9.05)	.97
Mus m 1, bedroom (μg/g)	0.42 (0.10-2.65)	0.60 (0.09-3.18)	.71
Der p 1, living room (μg/g)	0.44 (0.44-0.44)	0.44 (0.44-0.44)	.65
Der f 1, living room (μg/g)	0.27 (0.26-0.29)	0.28 (0.26-0.30)	.06
Fel d 1, living room (μg/g)	0.40 (0.23-1.92)	0.38 (0.19-0.86)	.29
Can f 1, living room (μg/g)	0.00 (0.00-0.06)	0.00 (0.00-0.08)	.87
Bla g 1, living room (U/g)	0.76 (0.20-5.32)	0.97 (0.20-7.60)	.95
Mus m 1, living room (μg/g)	0.46 (0.10-2.35)	0.58 (0.09-2.10)	.94

Values are presented as medians (interquartile ranges), mean ± standard deviation, or percentages (numbers). Mann-Whitney *U* or Pearson χ^2 tests were performed to compare numeric data or proportions between the 2 groups. Sample sizes are 446 for the bedroom dust and 378 for the living room dust. Sample size is 556 for child's race/ethnicity.

TABLE E4. Association between aeroallergen sensitization to specific allergens at year 3 and living room exposure to that allergen at year 1 and over all 3 years

	Year 1 exposure		Cumulative exposure (years 1-3)	
Allergen	**No.**	**Odds ratio (95% CI)**	**No.**	**Odds ratio (95% CI)**
Cockroach	307	1.01 (0.99-1.02)	271	1.47 (1.00-2.16)
Mouse	317	0.99 (0.96-1.02)	269	1.52 (1.06-2.17)
Dog	309	1.00 (0.99-1.01)	266	1.23 (0.84-1.80)
Dermatophagoides farinae (mite)	316	1.00 (0.95-1.06)	269	1.68 (1.06-2.65)
Dermatophagoides pteronyssinus (mite)	308	1.39 (1.02-1.89)	266	1.62 (0.65-4.04)
Cat	310	1.00 (0.99-1.01)	268	1.27 (0.95-1.70)

Note: To compare between columns, participants who did not have year 1 exposure data for individual allergens were eliminated from the models for cumulative exposure.

TABLE E5. Relationship between year 1 exposures and recurrent wheeze at age 3 years for microbiota cohort

Allergen exposure index	Correlation with recurrent wheeze
Bedroom	0.72 (0.55-0.93), $P = .01$
Living room	0.73 (0.59-0.92), $P = .01$

All models were adjusted for race, sex, sensitivity at age 3 years, perceived stress, and smoking.

TABLE E6. Taxa exhibiting a significant change in relative abundance across outcome groups

Phylum	Family	Genus	Species	Taxon ID	Atopic vs Neither (FDR < 0.05)	Both vs Neither (FDR < 0.05)
Acidobacteria	Acidobacteriaceae	Unclassified	Unclassified	2309		0.0421
Actinobacteria	Bifidobacteriaceae	*Bifidobacterium*	Unclassified	1863	0.0348	
Bacteroidetes	Bacteroidaceae	*Bacteroides*	Unclassified	2279		0.0472
Bacteroidetes	Blattabacteriaceae	*Blattabacterium*	Unclassified	1223		0.0421
Bacteroidetes	Blattabacteriaceae	*Blattabacterium*	Unclassified	1742		0.0472
Bacteroidetes	Porphyromonadaceae	*Dysgonomonas*	Unclassified	2282		0.0456
Bacteroidetes	Porphyromonadaceae	*Parabacteroides*	*Parabacteroides gordonii*	1392		0.0472
Bacteroidetes	Prevotellaceae	*Prevotella*	*Prevotella copri*	98		0.006
Bacteroidetes	Prevotellaceae	*Prevotella*	Unclassified	360		0.0203
Bacteroidetes	Prevotellaceae	*Prevotella*	Unclassified	361		0.0053
Bacteroidetes	**Prevotellaceae**	***Prevotella***	**Unclassified**	**362**	**0.0478**	**0.0053**
Bacteroidetes	Prevotellaceae	*Prevotella*	Unclassified	415		0.0399
Bacteroidetes	Prevotellaceae	*Prevotella*	Unclassified	455		0.0405
Bacteroidetes	Prevotellaceae	*Prevotella*	Unclassified	652		0.0421
Bacteroidetes	Prevotellaceae	*Prevotella*	Unclassified	1070		0.006
Bacteroidetes	Prevotellaceae	*Prevotella*	Unclassified	1391		0.0136
Bacteroidetes	**Prevotellaceae**	***Prevotella***	**Unclassified**	**1395**	**0.0478**	**0.0048**
Bacteroidetes	Prevotellaceae	*Prevotella*	Unclassified	1708		0.0089
Bacteroidetes	**Prevotellaceae**	***Prevotella***	**Unclassified**	**1709**	**0.0478**	**0.0089**
Bacteroidetes	Prevotellaceae	*Prevotella*	Unclassified	1710		0.0185
Bacteroidetes	**Prevotellaceae**	***Prevotella***	**Unclassified**	**1946**	**0.0319**	**0.006**
Bacteroidetes	Prevotellaceae	*Prevotella*	Unclassified	2085		0.042
Bacteroidetes	Prevotellaceae	*Prevotella*	Unclassified	2087		0.006
Bacteroidetes	RikenellaceaeII	Unclassified	Unclassified	456		0.0375
Bacteroidetes	RikenellaceaeII	Unclassified	Unclassified	556		0.0408
Bacteroidetes	RikenellaceaeII	Unclassified	Unclassified	1071		0.0456
Bacteroidetes	RikenellaceaeII	Unclassified	Unclassified	1072		0.0421
Bacteroidetes	RikenellaceaeII	Unclassified	Unclassified	1229		0.0494
Bacteroidetes	RikenellaceaeII	Unclassified	Unclassified	1231		0.0472
Bacteroidetes	RikenellaceaeII	Unclassified	Unclassified	1340		0.0252
Bacteroidetes	RikenellaceaeII	Unclassified	Unclassified	2277		0.0494
Bacteroidetes	RikenellaceaeII	Unclassified	Unclassified	2278		0.0421
Firmicutes	Catabacteriaceae	Unclassified	Unclassified	801		0.0421
Firmicutes	Enterococcaceae	*Melissococcus*	*Melissococcus plutonius*	903		0.0472
Firmicutes	Lachnospiraceae	*Blautia*	*Blautia producta*	842		0.0493
Firmicutes	Lachnospiraceae	*Clostridium*	Unclassified	431		0.0448
Firmicutes	Lachnospiraceae	*Clostridium*	Unclassified	1719		0.0494
Firmicutes	Lachnospiraceae	*Clostridium*	Unclassified	1984		0.0472
Firmicutes	**Lachnospiraceae**	***Coprococcus***	**Unclassified**	**975**	**0.0478**	**0.0399**
Firmicutes	Lachnospiraceae	*Roseburia*	*Roseburia faecis*	766	0.0319	
Firmicutes	Lachnospiraceae	Unclassified	Unclassified	57		0.0375
Firmicutes	Lachnospiraceae	Unclassified	Unclassified	181		0.0421
Firmicutes	Lachnospiraceae	Unclassified	Unclassified	590	0.0319	
Firmicutes	Lachnospiraceae	Unclassified	Unclassified	660		0.0091
Firmicutes	Lachnospiraceae	Unclassified	Unclassified	663		0.0456
Firmicutes	Lachnospiraceae	Unclassified	Unclassified	664	0.0478	
Firmicutes	Lachnospiraceae	Unclassified	Unclassified	665		0.0375
Firmicutes	**Lachnospiraceae**	**Unclassified**	**Unclassified**	**666**	**0.0348**	**0.0428**
Firmicutes	Lachnospiraceae	Unclassified	Unclassified	973		0.0428
Firmicutes	Lachnospiraceae	Unclassified	Unclassified	1139		0.0428
Firmicutes	Lachnospiraceae	Unclassified	Unclassified	1304		0.0456
Firmicutes	Lachnospiraceae	Unclassified	Unclassified	1305		0.0252
Firmicutes	Lachnospiraceae	Unclassified	Unclassified	1412		0.0421
Firmicutes	Lachnospiraceae	Unclassified	Unclassified	1442		0.0456
Firmicutes	Lachnospiraceae	Unclassified	Unclassified	1666		0.0458
Firmicutes	Lachnospiraceae	Unclassified	Unclassified	1729		0.05
Firmicutes	Lachnospiraceae	Unclassified	Unclassified	1731		0.0472
Firmicutes	Lachnospiraceae	Unclassified	Unclassified	1983		0.0461
Firmicutes	Lachnospiraceae	Unclassified	Unclassified	2055		0.0114
Firmicutes	Lachnospiraceae	Unclassified	Unclassified	2058		0.0494
Firmicutes	Lachnospiraceae	Unclassified	Unclassified	2059		0.0456
Firmicutes	Ruminococcaceae	*Faecalibacterium*	Unclassified	90		0.0456

(Continued)

TABLE E6. *(Continued)*

Phylum	Family	Genus	Species	Taxon ID	Atopic vs Neither (FDR < 0.05)	Both vs Neither (FDR < 0.05)
Firmicutes	Ruminococcaceae	*Faecalibacterium*	Unclassified	2054		0.0411
Firmicutes	Ruminococcaceae	*Oscillospira*	Unclassified	1850		0.0431
Firmicutes	Ruminococcaceae	*Ruminococcus*	*Ruminococcus bromii*	1052		0.0472
Firmicutes	Ruminococcaceae	Unclassified	Unclassified	274		0.0494
Firmicutes	Ruminococcaceae	Unclassified	Unclassified	707	0.0319	
Firmicutes	Ruminococcaceae	Unclassified	Unclassified	711		0.0428
Firmicutes	Ruminococcaceae	Unclassified	Unclassified	987		0.0273
Firmicutes	Ruminococcaceae	Unclassified	Unclassified	1561		0.0273
Firmicutes	Ruminococcaceae	Unclassified	Unclassified	1662		0.0488
Firmicutes	Ruminococcaceae	Unclassified	Unclassified	1765		0.042
Firmicutes	**Veillonellaceae**	***Phascolarctobacterium***	**Unclassified**	**1996**	**0.0348**	**0.0056**
Firmicutes	Veillonellaceae	Unclassified	Unclassified	527		0.0091
Firmicutes	Veillonellaceae	Unclassified	Unclassified	818		0.0288
Firmicutes	Veillonellaceae	Unclassified	Unclassified	1860		0.0421
Firmicutes	Unclassified	Unclassified	Unclassified	632		0.0428
Gemmatimonadetes	Gemmatimonadaceae	Unclassified	Unclassified	2205		0.0456
Planctomycetes	Planctomycetaceae	Unclassified	Unclassified	1565		0.0428
Proteobacteria	Desulfovibrionaceae	Unclassified	Unclassified	524		0.0494
Synergistetes	Dethiosulfovibrionaceae	TG5	Unclassified	2124	0.0478	
Tenericutes	Erysipelotrichaceae	ATCC_27806	*Eubacterium biforme*	748		0.0421
Tenericutes	Erysipelotrichaceae	ATCC_27806	*Eubacterium biforme*	1807		0.0428
Tenericutes	Erysipelotrichaceae	*Bulleidia*	p-1630-c5	1866		0.042
Tenericutes	Erysipelotrichaceae	*Catenibacterium*	*Catenibacterium mitsuokai*	412		0.0089
Tenericutes	Erysipelotrichaceae	*Catenibacterium*	Unclassified	127		0.0178
Tenericutes	Erysipelotrichaceae	*Catenibacterium*	Unclassified	1967		0.0089
Tenericutes	**Unclassified**	**Unclassified**	**Unclassified**	**1743**	**0.0319**	**0.0053**
Total no. of significant taxa					14	82

Taxa shown in boldface are significantly enriched across both comparisons.
FDR, False discovery rate.

TABLE E7. Discriminatory taxa that exhibit a significant correlation with Mus m 1 or Bla g 1 allergen concentrations

				Mus m 1		Bla g 1	
eOTU	**Phylum**	**Family**	**Genus**	***r***	***FDR***	***r***	***FDR***
2279	Bacteroidetes	Bacteroidaceaea	*Bacteroides*	0.3	0.0473	0.31	0.0589
1742	Bacteroidetes	Blattabacteriaceae	*Blattabacterium*	0.1	0.597	0.61	0.000
1223	Bacteroidetes	Blattabacteriaceae	*Blattabacterium*	0.11	0.5528	0.7	0.000
2282	Bacteroidetes	Porphyromonadaceae	*Dsygonomonas*	0.18	0.3334	0.57	0.000
1392	Bacteroidetes	Porphyromonadaceae	*Parabacteroides*	0.36	0.0077	0.67	0.000
455	Bacteroidetes	Prevotellaceae	*Prevotella*	0.37	0.0052	0.27	0.144
1710	Bacteroidetes	Prevotellaceae	*Prevotella*	0.31	0.0361	0.23	0.2376
2087	Bacteroidetes	Prevotellaceae	*Prevotella*	0.29	0.0598	0.33	0.0411
1708	Bacteroidetes	Prevotellaceae	*Prevotella*	0.32	0.026	0.24	0.2299
456	Bacteroidetes	Rikenellaceae	Unclassified	0.38	0.0029	0.32	0.0455
1071	Bacteroidetes	Rikenellaceae	Unclassified	0.38	0.0036	0.33	0.0369
2277	Bacteroidetes	Rikenellaceae	Unclassified	0.32	0.026	0.23	0.2521
1072	Bacteroidetes	Rikenellaceae	Unclassified	0.27	0.1059	0.34	0.0315
556	Bacteroidetes	Rikenellaceae	Unclassified	0.36	0.0077	0.24	0.2242
1340	Bacteroidetes	Rikenellaceae	Unclassified	0.35	0.0099	0.3	0.0743
903	Firmicutes	Enterococcaceae	*Melissococcus*	0.05	0.793	0.37	0.0085
1412	Firmicutes	Lachnospiraceae	Unclassified	0.3	0.0427	0.08	0.8021
1850	Firmicutes	Ruminococcaceae	*Oscillospira*	0.31	0.0389	0.16	0.5359
818	Firmicutes	Veillonellaceae	Unclassified	0.3	0.0466	0.2	0.3426
632	Firmicutes	Unclassified	Unclassified	0.3	0.0423	0.06	0.8598
524	Proteobacteria	Desulfovibrioaceae	Unclassified	0.14	0.44	0.69	0.000
Total no. of significant taxa					14		10

eOTU, Empiric Operational Taxonomic Unit; *FDR*, false discovery rate.