Early-life home environment and risk of asthma among inner-city children

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Background: Environmental exposures in early life appear to play an important role in the pathogenesis of childhood asthma, but the potentially modifiable exposures that lead to asthma remain uncertain.

Objective: We sought to identify early-life environmental risk factors for childhood asthma in a birth cohort of high-risk inner-city children.

Methods: We examined the relationship of prenatal and early-life environmental factors to the occurrence of asthma at 7 years of age among 442 children.

Results: Higher house dust concentrations of cockroach, mouse, and cat allergens in the first 3 years of life were associated with lower risk of asthma (for cockroach allergen: odds ratio per interquartile range increase in concentration, 0.55; 95% CI, 0.36-0.86; $P < .01$). House dust microbiome analysis using 16S ribosomal RNA sequencing identified 202 and 171 bacterial taxa that were significantly (false discovery rate < 0.05) more or less abundant, respectively, in the homes of children with asthma. A majority of these bacteria were significantly correlated with 1 of more allergen concentrations. Other factors associated significantly positively with asthma included umbilical cord plasma cotinine concentration (odds ratio per geometric SD increase in concentration, 1.76; 95% CI, 1.00-3.09; $P = .048$) and maternal stress and depression scores.

Conclusion: Among high-risk inner-city children, higher indoor levels of pet or pest allergens in infancy were associated with lower risk of asthma. The abundance of a number of bacterial taxa in house dust was associated with increased or decreased asthma risk. Prenatal tobacco smoke exposure and higher maternal stress and depression scores in early life were associated with increased asthma risk. (J Allergy Clin Immunol 2017;■■■:■■■-■■■.)

Key words: *Asthma, environment, allergy, allergen, microbiome, stress, depression, smoking*

Asthma is the most common chronic disease of childhood[1] and is responsible for substantial morbidity and health care costs. Environmental exposures in early life appear to play an important role in the pathogenesis of childhood asthma, but more information is needed to identify specific exposures that could potentially be modified to prevent asthma. Childhood asthma is associated

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Abbreviations used
FDR: False discovery rate
URECA: Urban Environment and Childhood Asthma Study

with sensitization to inhalant allergens,[2,3] whereas the effect of early-life exposure to these allergens or their sources on asthma risk is unclear[3,4] and might differ among allergens.[5] Children exposed to farm animals in the early postnatal period have a lower risk of allergies and asthma,[6] and a similar effect of early exposure to pets has been observed in some but not all studies.[7,8] Increased early-life exposure to microbial products, such as bacterial endotoxin, appears to be associated with reduced susceptibility to atopic asthma in childhood.[9,10] A recent study demonstrated that the gastrointestinal microbiome of 3-month-old children with wheezing and atopy at age 1 year differed from that of children with neither wheezing nor atopy.[11] Homes with pet dogs have a distinct microbiome,[12] and mice orally supplemented with house dust from homes with dogs are protected against ovalbumin- or cockroach allergen–mediated airway pathology.[13] Other potentially modifiable factors that might be linked to asthma risk include psychosocial stress, nutritional factors, and exposure to pollutants, such as tobacco smoke.

The Urban Environment and Childhood Asthma (URECA) study[14] was designed to investigate risk factors for asthma in a birth cohort of US high-risk inner-city children, a population with a high burden of asthma morbidity and a distinct environment.[15] In this cohort exposure to higher levels of cockroach, mouse, and cat allergen in the home in the first few months of life was associated with a lower risk of recurrent wheezing at 3 years of age, and the microbial composition of house dust in early life was related to the later development of wheezing and allergic sensitization.[16] In this report we examine exposures in the prenatal period and first 3 years of life, including allergens and microbes in house dust, as potential risk factors for asthma at age 7 years.

METHODS

Study design and participants

The URECA study is a birth cohort study initiated in 2005 in inner-city Baltimore, Boston, New York City, and St Louis.[14] Pregnant women aged 18 years or older were recruited with selection criteria, including a history of asthma, allergic rhinitis, or eczema, in the mother or father; for full entry criteria, see the Methods section in this article's Online Repository at www.jacionline.org). Informed consent was obtained from the women enrolled and, after birth, from the parent or legal guardian of the infant.

Data collection

Maternal questionnaires, including those on smoking, stress, and depression, were administered prenatally and annually after the child's birth. Postnatal child health questionnaires were administered to a parent every 3 months through age 7 years. Annual visits of the child and parent to the study center starting at 1 year of age included questionnaires, anthropomorphic measurements, and phlebotomy. Questionnaires included the Perceived Stress Scale,[17] the Edinburgh Postpartum Depression Scale,[18] and additional questionnaires to assess stress related to neighborhood factors, violence, and economic hardship.[19] Parent-reported colds were ascertained by means of telephone questionnaire every 3 months throughout the first 3 years of life, and the number of colds within each of the first 3 years was analyzed as a potential predictor of asthma at age 7 years.

Levels of allergen-specific IgE (ImmunoCAP; Phadia, Uppsala, Sweden) for milk, egg, peanut, and German cockroach were measured yearly until age 3 years and then at the ages of 5 and 7 years. Levels of specific IgE for dust mites, dog, cat, mouse, and *Alternaria* species were measured at 2, 3, 5, and 7 years of age. Skin prick testing was performed at 3 years of age and again at 5 and 7 years of age for 14 common aeroallergens (listed in the Methods section in this article's Online Repository). Aeroallergen sensitization was defined as a wheal of 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.

At age 7 years, spirometry was performed in accordance with American Thoracic Society guidelines[20] and repeated after 4 inhalations of albuterol hydrofluoroalkane metered-dose inhaler (90 μg per inhalation). At a separate visit, methacholine challenge (details are provided in the Methods section in this article's Online Repository) was performed.

Home visits to collect environmental data and specimens began after birth, with visits 3 months after birth and in the second and third years of life that included house dust collection (details are provided in the Methods section in this article's Online Repository). Dust samples were 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 means of ELISA (Indoor Biotechnologies, Charlottesville, Va). Dust samples were also assayed for ergosterol, a fungal membrane lipid, by using gas chromatography–mass spectroscopy, and for endotoxin, a bacterial cell wall constituent, by using the recombinant factor C assay. When sufficient sample remained after allergen analysis, dust specimens collected at 3 months of age underwent culture-independent microbiome profiling using 16S rRNA sequencing (details are provided in the Methods section in this article's Online Repository). Airborne nicotine concentration, a surrogate for environmental tobacco smoke concentration, was measured with a passive diffusion filter exposed for 14 days. Indoor nitrogen dioxide concentration was measured during the same 14 days with a modified diffusion filter sampler (Ogawa Sampler; Ogawa & Company USA, Pompano Beach, Fla).

Primary outcome

The prespecified primary study outcome was asthma at age 7 years. Children were classified as having asthma at age 7 years if at least 1 of 3 conditions was met (see Table E1 in this article's Online Repository at www.jacionline.org): (1) a parent-reported physician's diagnosis of asthma between age 4 and 7 years combined with asthma symptoms or the use of asthma controller medication for 6 of the past 12 months; (2) a methacholine PC_{20} value of 4 mg/mL or less or albuterol-induced FEV_1 reversibility of 10% or more combined with asthma symptoms or use of asthma controller medication for 6 of the past 12 months; or (3) report in the past 12 months of 2 or more wheezing episodes, 2 or more doctor's office visits for asthma/wheeze, 1 or more hospitalizations for asthma/wheeze, or use of controller medications for 6 of the past 12 months.

Statistical analysis

We used multiple imputation by chained equations[21,22] to impute missing data (approximately 10%; details are provided in the Methods section in this article's Online Repository). An allergen exposure index was derived for Bla g 1, Mus m 1, Fel d 1, and Can f 1, the 4 allergens that were significantly or nearly significantly associated with the development of asthma, by summing the quartiles (0, 1, 2, or 3) of exposure levels for each allergen (potential range, 0-12; details are provided in the Methods section in this article's Online Repository).

Relationships of demographic, perinatal, and family factors to asthma at age 7 years were assessed by using logistic regression. The relationships of environmental exposures in early life to asthma at age 7 years were assessed by means of logistic regression, with adjustment for sex, race, maternal asthma, and (except for models of stress and depression as risk factors) maternal Perceived Stress Scale score. Allergen and other environmental measurement data were log-transformed before analysis.

Microbiota data were analyzed in QIIME.[23] The relative abundance of bacterial operational taxonomic units in house dust at 3 months of age was

TABLE I. Characteristics of study population and unadjusted associations of these characteristics with asthma at age 7 years

	No.	Overall (n = 442)*	No asthma (n = 312)*	Asthma (n = 130)*	Unadjusted OR (95% CI)	*P* value
Mother's education						
Less than high school	441	183 (42%)	129 (42%)	54 (41%)	Reference	.99
High school		151 (34%)	107 (34%)	44 (34%)	0.98 (0.61-1.58)	
More than high school		107 (24%)	75 (24%)	32 (25%)	1.02 (0.60-1.72)	
Annual income <$15,000	441	304 (69%)	212 (68%)	92 (71%)	1.13 (0.72-1.78)	.67
Mother is married	441	60 (14%)	44 (14%)	16 (12%)	0.86 (0.45-1.56)	.72
Child's race						
Black	442	318 (72%)	221 (71%)	97 (75%)	Reference	.68
Hispanic		87 (20%)	63 (20%)	24 (18%)	0.87 (0.51-1.46)	
Mixed/other		37 (8%)	28 (9%)	9 (7%)	0.74 (0.32-1.58)	
Child's gestational age (wk)	442	38.8 ± 1.50	38.9 ± 1.47	38.6 ± 1.54	0.90 (0.79-1.04)	.15
Child's birth weight (g)†	442	3249 ± 483	3265 ± 478	3212 ± 496	0.80 (0.52-1.22)	.30
Cesarean section delivery	442	135 (31%)	91 (29%)	44 (34%)	1.24 (0.80-1.92)	.39
Mother breast-fed	427	240 (56%)	169 (56%)	71 (57%)	1.06 (0.70-1.62)	.86
Age of mother at time of birth (y)	442	24.4 ± 5.95	24.3 ± 5.73	24.6 ± 6.46	1.01 (0.98-1.05)	.57
Child's sex: male	442	226 (51%)	158 (51%)	68 (52%)	1.07 (0.71-1.61)	.83
Mother has had asthma	440	221 (50%)	143 (46%)	78 (60%)	1.79 (1.18-2.74)	.01
Mother is atopic	426	295 (69%)	199 (67%)	96 (74%)	1.43 (0.90-2.30)	.16
Father has had asthma	400	120 (30%)	79 (28%)	41 (35%)	1.39 (0.87-2.20)	.20
No. of hours/week of day care in first year‡	423	11.8 (18.1)	12.0 (18.0)	11.4 (18.3)	1.00 (0.99-1.01)	.77
No. of children in home§	441	2.3 (1.45)	2.3 (1.43)	2.3 (1.50)	1.01 (0.86-1.19)	.90
Recurrent wheeze at age 3 y	433	151 (35%)	67 (22%)	84 (67%)	7.1 (4.5-11.4)	<.01

*Values are counts (percentages) or means ± SDs.
†Odds ratio for child's birth weight represents the odds for a 1000-g higher birth weight.
‡Odds ratio for number of hours of day care represents the odds for 1 hour per week more day care.
§Odds ratio for number of children in home represents the odds for each additional child in the home.

compared between the homes of children who did and did not later have asthma by using 3 models (Poisson, negative binomial, and zero-inflated negative binomial regression models) and the Bayesian information criterion to determine the most appropriate model for each specific operational taxonomic unit.[24] Benjamini-Hochberg false discovery correction was used, and a corrected *P* value of less than .05 was considered significant.

RESULTS

Participants' characteristics

Between February 2005 and March 2007, 1850 families were screened, 776 met eligibility criteria, and 560 newborns were enrolled at birth (see Fig E1 in this article's Online Repository at www.jacionline.org). Of those enrolled, 442 (79%) had outcome data to classify asthma status at age 7 years, and 130 (29%) of these children had asthma. Most children classified as having asthma met 2 or more of the criteria for this diagnosis (see Fig E2 in this article's Online Repository at www.jacionline.org). Among children who underwent allergy testing at 6 or 7 years of age, 90 of 129 children with asthma and 179 of 307 children without asthma were sensitized to 1 or more aeroallergens (70% and 58%, respectively; $P = .025$; see Table E2 in this article's Online Repository at www.jacionline.org).

Family history, perinatal factors, early sensitization, and asthma

Children whose mothers reported having asthma were more likely to have asthma at age 7 years (odds ratio, 1.79; 95% CI, 1.18-2.74; $P = .01$; Table I). Other demographic factors and perinatal history were not associated with asthma occurrence (Table I). As expected, recurrent wheezing at 3 years of age was associated with asthma at age 7 years, although there was only partial concordance between these phenotypes (Table I). Sensitization to more than 1 aeroallergen at age 2 and 3 years was significantly associated with the development of asthma (see Fig E3 in this article's Online Repository at www.jacionline.org).

Allergen exposure and asthma

For the aeroallergens Bla g 1, Mus m 1, and Fel d 1, higher allergen concentrations in house dust summed over those of the specimens collected at 3 months, 2 years, and 3 years of age (defined as the cumulative allergen concentration) was associated with lower risk of asthma at age 7 years, with odds ratios of 0.55 (95% CI, 0.36-0.86; $P < .01$), 0.68 (95% CI, 0.49-0.94; $P = .02$), and 0.71 (95% CI, 0.51-1.00; $P = .05$), respectively, for a cumulative allergen concentration higher by 1 interquartile range (Table II). A nonsignificant association in the same direction was observed for the dust concentration of Can f 1. Higher levels of these 4 allergens at 3 months of age, as quantified by the allergen exposure index, was associated with significantly lower risk of asthma, with the odds ratio per 1 interquartile range higher allergen exposure index of 0.59 (95% CI, 0.44-0.80; $P < .001$). The association of higher allergen exposure index at age 3 months with lower risk of asthma was observed among all participants ($P = .008$; Fig 1, *A*) and among both children sensitized ($P = .02$) and not sensitized ($P = .02$) to 1 or more of these allergens at age 3 years (Fig 1, *B*). Similar associations were observed for cumulative allergen exposure during the first 3 years of life (Fig 1, *C* and *D*, and Table II).

Microbial exposure and asthma

There was sufficient dust sample for microbiome profiling of 248 (56%) of the 442 children's homes. This subsample was

TABLE II. Association between home dust allergen concentration in the first 3 years of life and asthma at 7 years of age

	Allergen concentration*			
	No asthma (n = 312)	Asthma (n = 130)	Adjusted OR (95% CI)†	*P* value
Cockroach (Bla g 1 [U/g])				
Age 3 mo	1.20 (0.20-7.15)	0.20 (0.20-3.70)	0.69 (0.45-1.06)	.09
Cumulative‡	7.00 (0.59-36.7)	2.19 (0.59-15.2)	0.55 (0.36-0.86)	<.01
Mouse (Mus m 1 [μg/g])				
Age 3 mo	0.57 (0.13-2.80)	0.22 (0.07-2.52)	0.75 (0.55-1.01)	.06
Cumulative‡	4.29 (0.71-19.0)	3.66 (0.28-11.5)	0.68 (0.49-0.94)	.02
Dust mite (Der f 1 [μg/g])				
Age 3 mo	0.28 (0.26-0.33)	0.27 (0.26-0.30)	0.98 (0.91-1.04)	.46
Cumulative‡	0.85 (0.80-1.15)	0.83 (0.79-1.16)	0.94 (0.82-1.09)	.42
Dog (Can f 1 [μg/g])				
Age 3 mo	0.005 (0.005-0.050)	0.005 (0.005-0.005)	0.62 (0.37-1.03)	.06
Cumulative‡	0.046 (0.015-0.684)	0.015 (0.015-0.265)	0.78 (0.56-1.09)	.15
Cat (Fel d 1 [μg/g])				
Age 3 mo	0.36 (0.20-1.33)	0.28 (0.19-0.59)	0.78 (0.62-0.98)	.03
Cumulative‡	1.17 (0.64-9.79)	0.94 (0.64-2.86)	0.71 (0.51-1.00)	.05
Sum of exposures index§				
Age 3 mo	3.99 ± 2.29	3.16 ± 2.28	0.59 (0.44-0.80)	<.001
Cumulative‡	11.33 ± 5.63	8.95 ± 5.05	0.52 (0.36-0.74)	<.001

*Values are medians (interquartile ranges), except for sum of exposures index values, which are means ± SDs.
†Odds ratios are from a logistic regression model controlling for sex, race, maternal Perceived Stress Scale, and maternal asthma averaged across 8 imputed data sets and represent the odds for an interquartile increase in exposure. Can f 1 is an exception; because of a highly skewed distribution, the odds ratio for Can f 1 is for an increase from the 25th to the 85th percentile.
‡Cumulative exposure is defined as the sum of allergen concentrations at age 3 months, 2 years, and 3 years.
§Sum of exposures is calculated by combining cat, mouse, cockroach, and dog into a single index based on quartiles of exposure to individual allergens, resulting in a range of 0 to 12.

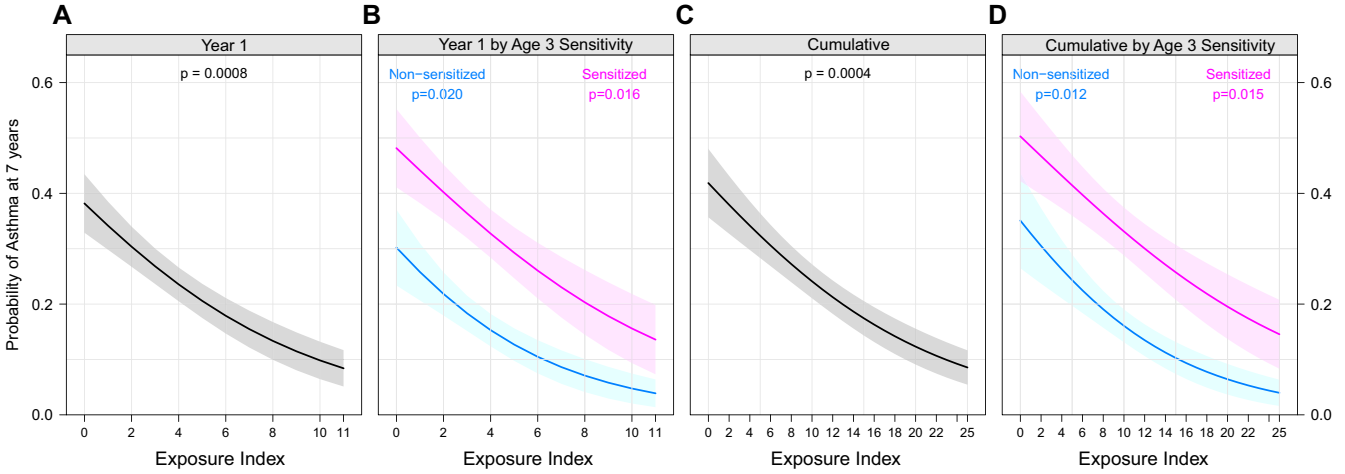


FIG 1. Allergen exposures and asthma relationships between exposure to specific allergens and asthma. The probability of asthma at age 7 years was determined by using logistic regression according to the sum of exposure tertiles (cockroach, mouse, cat, and dog). **A,** Sum of the exposures in the first year of life. **B,** Same as Fig 1, *A*, stratified by aeroallergen sensitization at age 3 years (pink = sensitive, blue = not sensitive). **C,** Cumulative sum of exposures across years 1, 2, and 3. **D,** Same as Fig 1, *C*, stratified by aeroallergen sensitization at age 3 years. *P* values are from a logistic regression model testing a relationship between exposure levels and asthma and stratified by sensitization in Fig 1, *B* and *D*.

similar to the remainder of the cohort, except for a slightly higher proportion with black race and a lower proportion with a paternal history of asthma (see Table E3 in this article's Online Repository at www.jacionline.org). Bacterial α- and β-diversity did not differ significantly between the homes of children who did or did not have asthma, nor did these diversity measures differ between the homes of the children who did or did not have atopy at age 7 years (see Fig E4 in this article's Online Repository at www.jacionline.org).

Taxon-based comparisons identified 202 and 171 bacterial taxa, representing a breadth of bacterial phyla, that were significantly (false discovery rate [FDR] < 0.05) more or less abundant, respectively, in the homes of children with asthma (Fig 2 and see Table E4 in this article's Online Repository at www.jacionline.org). When this analysis was adjusted for atopic status at 7 years of age, 57% of these 473 taxa remained significantly (FDR < 0.05) associated with the development of asthma at age 7 years, and more than 90% of the taxa had a regression

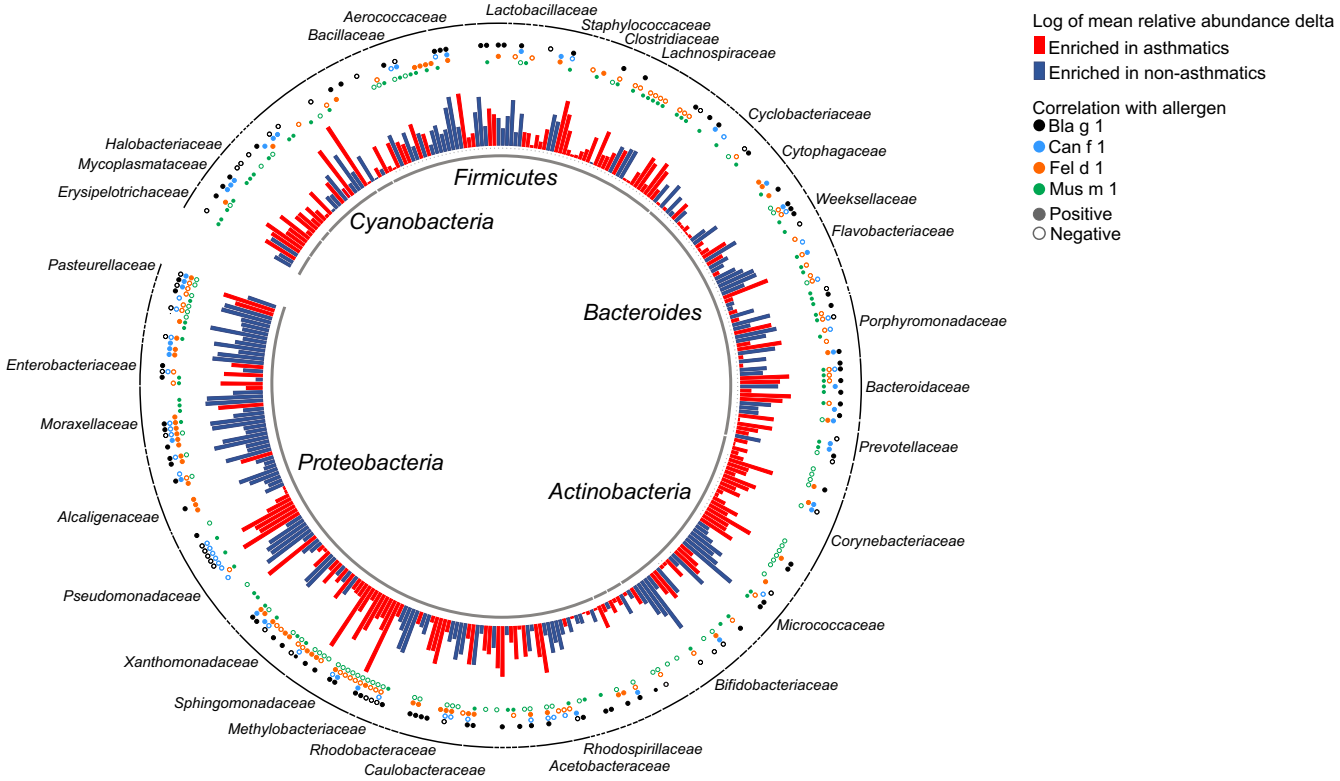


FIG 2. Classified bacterial taxa in house dust at 3 months of age in relation to simultaneous dust allergen concentrations and to asthma status at age 7 years. The 356 classified bacterial taxa (taxa classified as "mitochondria" or unclassified at the phylum level were omitted from this figure) that differed (FDR < 0.05) between the homes of asthmatic and nonasthmatic subjects are shown as *bars,* with color indicating whether the taxon was more abundant in the homes of asthmatic (red) or nonasthmatic (blue) subjects and bar height indicating the magnitude of between-group relative abundance delta. Bacterial taxa with abundance that was correlated with allergen levels are indicated by *circles above the bars,* with color indicating the allergen and *solid* or *open circles* indicating positive or negative correlation, respectively.

coefficient relating the abundance of the taxon to asthma outcome within 4% of the coefficient in the unadjusted analysis. Among those taxa most enriched in homes of children with asthma were potential pathogens, including *Staphylococcus*, *Haemophilus* (Pasturellaceae), *Corynebacterium*, and several *Sphingomonas* species members. In contrast, dust from the homes of children who did not have asthma was enriched in *Kocuria* (Micrococcaceae), *Alloiococcus* (Aerobacteriaceae), *Bifidobacterium*, and *Acinetobacter* (Moraxellaceae) species. Many of the bacterial taxa that differed significantly in abundance between the homes of children who did and did not have asthma also exhibited significant correlations with allergen concentrations (indicated by circles above bars in Fig 2). Of the 373 taxa that differed significantly between the 2 groups of homes, 34.6% had significant positive or negative correlations between bacterial abundance and Bla g 1 concentrations compared with only 14.7% of 373 randomly selected other taxa matched for distribution of abundance value ($P < .001$). Similar relationships were seen for Mus m 1, Fel d 1, and Can f 1 (21.4% vs 6.2%, 34.3% vs 12.6%, and 45.0% vs 11.0%, respectively; $P < .001$ for all).

We examined whether the association between higher house dust allergen concentration at age 3 months and lower risk of asthma at age 7 years might be mediated by the house dust microbiome. Mediation analysis was performed with the HIMA R package,[25] simultaneously assessing potential mediation by all 373 bacterial taxa that were present in differential abundance between the homes of children who did or did not have asthma. For cat, dog, and mouse allergens and the sum of exposure to these allergens plus cockroach allergen, we observed no evidence of statistically significant mediation. For cockroach allergen, we observed significant partial mediation by 2 bacterial taxa, *Bifidobacterium* and *Brevundimonas* species, which explained 13.4% ($P = .05$) and 12.1% ($P = .02$), respectively, of the association between higher cockroach allergen concentration and lower asthma risk. For these 2 bacterial taxa, higher abundance in house dust was associated with lower asthma risk.

Maternal smoking, stress, depression, and asthma

The umbilical cord plasma cotinine concentration was a significant predictor of asthma (adjusted odds ratio, 1.76 for an increase of 1 geometric SD in cotinine concentration; 95% CI, 1.00-3.09; $P = .048$), whereas reported maternal smoking (either prenatally or postnatally) and smoking by others in the home (assessed postnatally only) was not (Table III). Maternal stress and depression in the first 3 years of life were significantly associated with the development of asthma at age 7 years (Table III). For example, maternal depression score at age 3 years was significantly associated with asthma at age 7 years (adjusted odds ratio,

TABLE III. Association between early-life exposures and asthma at age 7 years

	No asthma*	Asthma*	Adjusted OR (95% CI)†	*P* value
Prenatal				
Mother smoked	53 (17%)	27 (21%)	1.25 (0.74-2.13)	.41
Umbilical cord plasma cotinine concentration (ng/mL)‡	3.14 ± 3.03	4.17 ± 4.17	1.76 (1.00-3.09)	.048
No. of smokers in home	0.8 ± 1.0	0.7 ± 0.8	0.83 (0.66-1.04)	.11
Maternal perceived stress§	5.4 ± 3.5	5.9 ± 3.3	1.05 (0.99-1.12)	.11
Maternal composite stress‖	6.0 ± 1.9	6.2 ± 1.9	1.05 (0.93-1.17)	.44
Maternal depression¶	7.6 ± 6.1	8.9 ± 6.6	1.03 (1.00-1.06)	.08
Year 1				
Mother smoked	131 (43%)	47 (37%)	0.74 (0.48-1.15)	.18
No. of smokers in home	1.3 ± 1.3	1.5 ± 2.0	1.04 (0.91-1.19)	.55
Nitrogen dioxide (ppb)#	20.4 (12.0-31.7)	20.0 (10.9-32.4)	0.97 (0.75-1.26)	.82
Nicotine (μg/g)#	0.4 (0.2-1.4)	0.4 (0.2-1.3)	0.98 (0.66-1.44)	.90
Ergosterol (ng/mg)#	3.7 (2.1-6.1)	3.1 (2.1-6.2)	0.84 (0.61-1.15)	.27
Endotoxin (EU/mg)#	38.7 (17.5-90.2)	29.5 (14.6-62.2)	0.74 (0.51-1.07)	.11
Maternal perceived stress§	4.4 ± 2.7	5.0 ± 2.8	1.07 (0.99-1.16)	.07
Maternal composite stress‖	7.7 ± 1.0	7.8 ± 0.9	1.14 (0.92-1.42)	.23
Maternal depression¶	5.6 ± 5.3	7.0 ± 6.5	1.04 (1.00-1.08)	.04
No. of colds	4.1 ± 2.9	4.8 ± 2.7	1.07 (0.99-1.15)	.09
Year 2				
Mother smoked	124 (41%)	47 (37%)	0.83 (0.53-1.30)	.42
No. of smokers in home	1.2 ± 1.2	1.3 ± 2.2	1.02 (0.89-1.17)	.77
Maternal perceived stress§	4.4 ± 2.9	5.3 ± 3.1	1.10 (1.02-1.18)	<.01
Maternal composite stress‖	6.0 ± 1.8	6.3 ± 1.9	1.07 (0.95-1.20)	.26
Maternal depression¶	5.7 ± 6.0	7.5 ± 6.8	1.04 (1.00-1.07)	.03
No. of colds	3.7 ± 2.7	5.2 ± 3.8	1.13 (1.06-1.22)	<.01
Year 3				
Mother smoked	125 (40%)	48 (38%)	0.80 (0.52-1.24)	.31
No. of smokers in home	1.1 ± 1.1	1.1 ± 1.4	0.99 (0.83-1.18)	.91
Endotoxin (EU/mg)#	32.0 (17.6-61.2)	32.9 (16.2-58.8)	0.86 (0.66-1.13)	.29
Maternal perceived stress§	4.5 ± 2.8	5.3 ± 2.9	1.10 (1.02-1.18)	.01
Maternal composite stress‖	6.0 ± 1.8	6.6 ± 1.8	1.16 (1.02-1.31)	.02
Maternal depression¶	5.2 ± 5.8	7.3 ± 6.9	1.05 (1.02-1.09)	<.01
No. of colds	3.5 ± 2.8	4.5 ± 3.0	1.11 (1.03-1.20)	<.01

*Values are counts (percentages), means ± SDs, or medians (interquartile ranges) from raw nonimputed data. The exception is umbilical cord plasma cotinine concentration, which is expressed as geometric mean ± geometric SD because of a highly skewed distribution with many values less than the detection limit.
†Odds ratios are from a logistic regression model controlling for sex, race, and maternal asthma averaged across 8 imputed data sets (n = 442 for all). Model for number of colds is also controlled for maternal stress within each year.
‡Odds ratio is per increase of 1 geometric SD in cord plasma cotinine concentration.
§Odds ratio is per unit increase in the maternal Perceived Stress Scale, which ranges from 0 to 4.
‖Odds ratio is per unit increase in the composite stress scale, as described in text, which ranges from 0 to 9.
¶Odds ratio per unit increase in the maternal Edinburgh Postpartum Depression Scale, which ranges from 0 to 30.
#Before inclusion in the model, nitrogen dioxide, nicotine, ergosterol, and endotoxin values were log-transformed (base 10), and their estimates represent the odds for an interquartile increase in exposure.

1.05 for each 1-unit increase in Edinburgh Postpartum Depression Score [range, 0-30]; 95% CI, 1.02-1.09; $P < .01$).

Other exposures and asthma

Indoor air nitrogen dioxide concentration and house dust concentrations of ergosterol and endotoxin were not associated with the development of asthma (Table III). The number of colds in the second and third years of life was associated with the development of asthma at age 7 years (Table III).

DISCUSSION

The URECA study evaluated the home environments of high-risk children in 4 economically disadvantaged urban neighborhoods beginning in the prenatal period to identify potentially modifiable risk factors for childhood asthma. Higher exposure to cockroach, mouse, and cat allergens during infancy was associated with a lower risk of asthma at age 7 years. The bacterial microbiota in house dust in the first year of life differed between the homes of children who did and did not have asthma, and a majority of the bacterial taxa positively or negatively associated with asthma risk were also correlated with concentrations of the indoor allergens associated with asthma risk. In addition, umbilical cord plasma cotinine concentrations and maternal stress and depression scores in early life were risk factors for asthma at age 7 years. Indoor nitrogen dioxide concentrations and house dust concentrations of the bacterial cell-wall constituent endotoxin and fungal membrane lipid ergosterol, which have been associated with asthma in some studies, were not associated with the development of asthma in this cohort.

These findings suggest that the early-life environment of children in the inner city might include exposures that increase asthma risk, such as prenatal tobacco smoke and maternal stress, and exposures that reduce risk, such as specific indoor allergens,

bacteria, or bacterial products. Exposure to environments that are rich in allergens and microbes have been associated with low rates of asthma and other allergic diseases.[2,26,27] For example, the environment of Amish farms in the United States is associated with a lower prevalence of asthma and allergen sensitization than Hutterite farms, and house dust from the former setting has a higher endotoxin concentration and distinct microbiota compared with the latter.[28] Compared with other environments, inner cities are extensively paved and might have a paucity of outdoor exposures to animals, plants, and sources of microbes, such as soil and green space. In this setting indoor pests or pets might provide biologic signals that promote normal lung and immunologic development. These findings extend our earlier report that exposure to cockroach, mouse, and cat allergens in infancy is associated with a lower risk of recurrent wheezing through age 3 years,[16] although it is important to note that there is incomplete concordance between the phenotypes of recurrent wheezing at age 3 years and asthma at age 7 years, with 35% of the children with asthma at age 7 years not having had recurrent wheezing at age 3 years. Relationships of allergen exposure to wheezing or asthma could depend on the nature of the allergen exposure and the other environmental factors present in a specific environment; for example, exposure to dust mite in the URECA study was not associated with asthma risk, whereas in another US study early-life dust mite exposure was associated with increased asthma risk.[29] Also, in contrast to our findings for cat allergen in US inner cities, a pooled analysis of data from 11 European birth cohorts found no association between the presence of cats in the home during infancy and asthma at school age, although those studies did not include measured allergen concentrations.[8]

One factor that might influence the association of higher early-life allergen exposure and lower asthma risk is a home bacterial microbiota altered by the presence of pets and pests. A number of bacterial taxa differed in abundance in the homes of children who did and did not have asthma, and most of these taxa were also significantly associated with cockroach, mouse, or cat allergens. Previous studies have demonstrated that dogs alter the home microbiota[12,30] and that microbes associated with dogs can reduce allergic airway inflammation and bronchial reactivity in mouse models.[13]

The characteristics of allergen-associated bacterial taxa in the URECA study might offer clues relevant to primary prevention strategies. Members of the *Kocuria* genus, which was more abundant in the homes of children who did not have asthma, are known to produce kocurin, a potent macrolide with activity against *Staphylococcus* species.[31] Also more abundant in the homes of children that did not have asthma were *Alloiococcus* species, the presence of which in the nasal microbiota is associated with a lower frequency of childhood upper respiratory tract infection,[32] and *Bifidobacterium* and *Acinetobacter*, species of which protect against allergic sensitization in murine models.[33,34] Bacterial taxa that were more abundant in the homes of children who had asthma included potential pathogens, such as *Staphylococcus*, *Haemophilus* (Pasturellaceae), *Corynebacterium*, and several *Sphingomonas* species members. In a Danish birth cohort colonization of the hypopharynx at age 1 month with *Haemophilus influenzae* was associated with increased risk of recurrent wheeze and asthma at age 5 years.[35] The observed associations between the abundance of specific bacterial taxa and asthma risk suggested that some of these associations might mediate the observed association of higher dust allergen concentrations with lower asthma risk. However, mediation analysis did not confirm such mediation, except in the case of cockroach allergen, for which 2 bacterial taxa, *Bifidobacterium* and *Brevundimonas* species, appeared to explain part of the inverse association between higher allergen concentration and lower asthma risk. Further research is needed to confirm and explore the potential mechanism of this observation.

The absence of significant differences in overall bacterial diversity in house dust between the homes of children who did or did not have asthma or atopy at age 7 years might suggest that specific bacterial taxa or communities have a greater effect on risk than overall diversity. Our earlier observation[16] that bacterial diversity was related to recurrent wheeze and atopy at age 3 years might reflect the difference of that phenotype from later-onset allergic sensitization by age 7 years; the use of 16S rRNA sequencing in this study, as opposed to a higher-resolution phylogenetic microarray in the earlier study; or the smaller number of subjects included in the earlier study.

Our findings confirm and extend previous studies linking prenatal smoke exposure and maternal stress and depression to childhood asthma. A meta-analysis of birth cohorts observed a modest association between questionnaire-reported prenatal smoking and asthma later in childhood.[36] Our finding that cord plasma cotinine levels predict asthma at age 7 years provides support for the role of prenatal smoking in the pathogenesis of asthma. Furthermore, the combination of maternal prenatal and postnatal stress has been associated with a higher risk of wheezing in early life.[37,38] It is unknown whether these associations are due to direct physiologic effects, such as altered hypothalamic-pituitary function and corticosteroid responsiveness, or to maternal behavioral responses to these conditions in their children.

The strengths of this study include a definition of asthma based on observations of a physician's diagnosis, symptoms, medication use, and lung physiology. The multicenter design helps to establish findings that are generalizable to high-risk urban children; however, a limitation is that relationships with environmental factors identified in the URECA study might be specific to this population and might depend on the personal or environmental context, including specific allergies, lifestyle, or neighborhood factors. Although the associations of bacterial taxa in house dust with the development of asthma were adjusted for multiple comparisons, replication in other study populations will be needed when such data become available. We did not measure the gastrointestinal or airway microbiome in early childhood, and therefore we cannot evaluate the independent effects of the house dust, airway, and gastrointestinal microbiomes. Another important caveat is that the observed associations do not necessarily establish causation and that, despite the prospective and longitudinal design of the study, with environmental assessments beginning at 3 months of age, it is possible that a family's health and health-related behaviors led to long-term alterations of home allergens, microbiome, and maternal stress, rather than these factors influencing health outcomes.

Our findings suggest that primary prevention strategies for childhood asthma in low-income urban communities should probably not focus on home allergen reduction and that exposure to a broad variety of proteins in early life might have health benefits with respect to asthma. Interventions to reduce prenatal smoking and reduce maternal stress and depression during pregnancy and infancy might hold promise for asthma prevention. Finally, our results in poor urban neighborhoods echo those in

farming communities, suggesting that early-life microbial exposures are associated with the risk of childhood asthma, findings that might provide targets for strategies to prevent asthma.

Key messages

- **Among urban children, higher indoor levels of pet- and pest-related allergens in infancy are associated with reduced risk of asthma at age 7 years.**
- **Maternal stress during infancy and prenatal maternal smoking are associated with increased asthma risk.**
- **The indoor bacterial microbiome in infancy also appears to be associated with asthma risk.**

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Early-life home environment and the risk of developing asthma among inner-city children: Online Repository

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METHODS

Full entry criteria for enrollment in the URECA study

Inclusion criteria

- Planning to deliver at the study hospital.
- A parental history of asthma, allergic rhinitis (hay fever), or eczema (atopic dermatitis). The presence of paternal allergy or asthma will be ascertained by maternal report.
- All study subjects will reside in census tracts with at least 20% of the residents with income below the poverty level.
- Gestational age at delivery of ≥ 34 weeks
- A suitable cord blood specimen must be obtained and processed to establish baseline cytokine secretion data.

Exclusion Criteria

- Respiratory distress requiring intubation and ventilation for four or more hours.
- Respiratory distress requiring either supplemental oxygen or CPAP for four or more days.
- Pneumonia requiring antibiotic treatment for one week or more.
- Significant congenital anomalies.
- Maternal HIV infection at time of delivery.
- Plans for the family to move out of the geographic area during the period of the study.
- Does not consent to all aspects of the study.
- Does not have access to a phone.
- Does not speak English (or Spanish at sites with Spanish-speaking staff).
- Administration of palivizumab (Synagis) for RSV prophylaxis.

House dust collection protocol

Dust specimens were collected when the baby was three months of age, and each year thereafter, following standard procedures at all study sites. A 10-minute dust sample was collected from the child's bed and bedroom floor using a vacuum and Mitest Dust Collector. A 5-minute sample was taken from the child's bedroom floor, and another 5-minute sample from the living room/family room seat. After

sample collection, the inner dust collectors was removed from the outer plastic housing and placed into separate sealable plastic bags for transport to the study center and shipment to the lab.

Allergy skin prick testing

Children were evaluated for sensitivity to the following 14 glycerinated extracts (Greer Laboratories): Dermatophagoides farinae, Dermatophagoides pteronyssinus, cat hair standardized, dog epithelia, mouse epithelia, rat epithelia, German cockroach, American/German cockroach mix, Alternaria tenuis, tree pollen (White Oak at St. Louis and Baltimore Sites; Birch Mix at Boston and New York sites), Aspergillus mix, Penicillium notatum, ragweed mix, and Timothy grass. Along with the allergens, a positive and negative control are tested.

Methacholine challenge testing

Methacholine airway responsiveness was measured by assessing the concentration of methacholine (Provocholine®) required to produce a drop in FEV_1 of 20% (PC_{20}) after the administration of increasing concentrations of methacholine using the small volume nebulizer-tidal breathing technique. The participant performed quiet resting (tidal) breathing while inhaling from a nebulizer, and then spirometry was performed. The procedure was repeated at 5-minute intervals with increasing concentrations of methacholine until a drop in FEV_1 of 20% occurred or the maximum concentration was given.

16S ribosomal RNA (rRNA) sequencing

Dust specimens collected at 3 months of age underwent culture-independent microbiome profiling using 16S ribosomal RNA (rRNA) sequencing. DNA was extracted as previously described [1,2] and the V4 region of the 16S rRNA gene amplified using primer pair F515 and R806 [3], with 12-base error

correction Golay barcoded reverse primers. [4] A total of 250 URECA samples were sequenced; 2 were omitted from subsequent analysis due to extremely low sequence reads.

Statistical analyses (additional details)

Due to the occurrence of missing data in the birth cohort (~10%), we used multiple imputation by chained equations (MICE)[5,6] to impute missing data, assuming both missing at random and missing completely at random. Eight imputed datasets were built using a fully conditional specification approach and a random forest method.[7] After the imputation stage, a logistic model was estimated for each of the 8 imputed datasets. In these models, the dependent variable was participant asthma status at age 7 (yes/no) and the independent variables were the socio-demographics, clinical characteristics and exposure during the first 3 years of life of the participants.

To examine the relationship between allergen exposures in the first three years of life and asthma at age 7 years, a relative importance analysis was done to determine which specific allergens showed the strongest association to asthma. Once this was established, an "allergen exposure index" was derived by taking into account the four most relevant allergens – bla g 1, mus m 1, fel d 1, and can f 1; the index was calculated by summing the quartiles (0, 1, 2, or 3) of exposure level for each allergen (potential range 0-12).

For microbiome analysis, a table of operational taxonomic units (OTUs) was generated from reconstructed paired end reads (generated using FLASH[16,]) which were demultiplexed, quality filtered (in QIIME[18]), multiply rarefied at a sequence depth of 173,841[8] and classified at 97% sequence similarity using USEARCH[4,9,10]. The final table contained a total of 51,094 OTUs. Alpha-diversity indices including richness, Pielou's evenness, Shannon, Inverse Simpson and Faith's phylogenetic diversity were calculated as previously described.[1] Bacterial beta-diversity was

analyzed and visualized using PCoA based on a weighted UniFrac distance matrix; Permutational Multivariate Analysis of Variance (PERMANOVA) was used to determine relationships between metadata variables and variation in community composition[11]. Bacterial taxa with abundance that differed between the homes of children who did and did not develop asthma were determined using a three-model approach (Poisson, negative binomial and zero-inflated negative binomial regression models) and Bayesian Information Criterion (BIC) to determine the most appropriate model for each OUT. [12] Benjamini-Hochberg false discovery correction was employed and a p-value >0.05 considered significant.

The SAS procedure MIANALYZE was applied to summarize parameter estimates with corresponding confidence intervals and p-values, which incorporated the resulting uncertainty associated with the multiple imputation used to obtain them. Allergen and bacterial abundance data were log-transformed prior to analysis. SAS 9.4 and R version 3.2.2 were utilized for analyses.

SUPPLEMENTAL TABLES AND FIGURES

Table E1. Definition of asthma at age 7 years in URECA Study

Asthma was considered to be present at age 7 years if at least one of the following

- Doctor diagnosis of asthma at some time between ages 4-7
  and one or more of the following occurred in the last 12 months:
  - ≥1 wheezing episodes or
  - ≥1 doctor visits for asthma/wheeze or
  - ≥1 hospitalization for asthma/wheeze or
  - Use of controller meds for 6 months

- Lung function criteria at age 7 years (reversibility ≥ 10% after administration of 4 puffs of albuterol OR methacholine PC20 ≤ 4 mg/mL)
  and one or more of the following occurred in the last 12 months:
  - ≥1 wheezing episodes or
  - ≥1 doctor visits for asthma/wheeze or
  - ≥1 hospitalization for asthma/wheeze or
  - Use of controller meds for 6 months

- Symptom-based criteria without doctor diagnosis:
  One or more of the following occurred in the last 12 months:
  - ≥2 wheezing episodes or
  - ≥2 doctor visits for asthma/wheeze or
  - ≥1 hospitalization for asthma/wheeze or
  - Use of controller meds for 6 months

Table E2. Clinical characteristics of participants by asthma status at age 7 years		
	No Asthma	Asthma
Atopy*	179/307 (58%)	90/129 (70%)
Increase in FEV_1 after bronchodilator $\geq$ 10%	65/193 (34%)	43/86 (50%)
Methacholine $PC_{20} \leq$ 4 mg/ml	94/237 (40%)	46/97 (47%)
FEV_1%predicted, mean (SD)	104.4 ± 14.4 (n = 265)	100.9 ± 15.5 (n = 115)
FEV_1/FVC ratio, mean (SD)	0.83 ± 0.07 (n = 193)	0.82 ± 0.08 (n = 86)
Asthma controller medication in past 12 months	14/312 (4%)	46/130 (35%)
Albuterol use in past 12 months	28/312 (9%)	103/129 (79%)
Prednisone in past 12 months	9/312 (3%)	35/130 (27%)

*Atopy was defined as a positive allergy skin test (wheal diameter $\geq$ 3mm great than glycerin control) or plasma specific IgE concentration $\geq$ 0.35 IU/mL to one or more aeroallergen. The difference between the no asthma and asthma groups was significant (p = 0.025). Other contrasts were not tested for statistical significance because they are part of the definition of asthma.

Table E3: Characteristics of participants with microbiome data compared to remainder of participants

	n	No microbiome data N=194	Microbiome cohort N=248	P-value
Mother's education: Less than high school	441	85 (44%)	98 (40%)	0.26
High school		58 (30%)	93 (38%)	
More than high school		50 (26%)	57 (23%)	
Annual income < $15K	441	129 (67%)	175 (71%)	0.46
Mother is married	441	26 (14%)	34 (14%)	0.99
Child's race: Black	442	129 (67%)	189 (76%)	0.03
Hispanic		49 (25%)	38 (15%)	
Mixed/other		16 (8%)	21 (8%)	
Child's gestational age (wks.)	442	38.8 ± 1.48	38.8 ± 1.51	0.70
Child's birthweight (g)	442	3264 ± 511	3237 ± 462	0.57
C-section delivery	442	63 (33%)	72 (29%)	0.50
Mother breastfed	427	105 (57%)	135 (56%)	0.83
Age of mother at time of birth	442	24.4 ± 6.16	24.4 ± 5.79	0.95
Child's gender: male	442	93 (48%)	133 (54%)	0.27
Mother has had asthma	440	103 (53%)	118 (48%)	0.29
Mother is atopic	426	136 (74%)	159 (66%)	0.09
Father has had asthma	400	62 (36%)	58 (26%)	0.03

*Values are counts (percentages) or means ± standard deviations.

Table E4. Bacterial taxa present in different abundance at age 3 months between the homes of children who did and did not asthma at age 7 years

[Excel spreadsheet. Separate file.]

Figure E1. CONSORT diagram

Figure E2.

The number of participants who met each of the 3 components that define asthma, as outlined in Table S1. For this figure, those criteria are labeled “doctor diagnosis,” “lung function,” and “symptoms.” The numbers outside the Venn diagram represent the total number of participants who met that specific criterion (not mutually exclusive), and the percentages inside the diagram show the percentage of participants who met one, two, or all three of the components.

Figure E3.

Relationship between sensitization to aeroallergens at ages 2 and 3 years and the development of asthma at age 7 years. Probability of asthma (95% CI indicated by line) is determined by logistic regression according to the number of sensitivities (0, 1, or 2+) at ages 2 and 3 years. Odds ratios (95% CIs) compare those with 1 and 2+ sensitivities to those with no sensitivities.

Figure E4.

Bacterial alpha-diversity (A) and beta-diversity (B) in the dust collected from the homes of children who did or did not develop asthma or atopy. No measures were significantly different between the homes of children who did and did not develop asthma at age 7, or between the homes of children who did or or did not develop atopy at age 3 or age 7.

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