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Cockroach allergen exposure and plasma cytokines among children in a tropical environment

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INTRODUCTION

Asthma is a major public health problem and the most common chronic non-communicable disease among children in the United States (U.S.) (1). In this country, the burden of asthma is unequally shared among racial or ethnic groups, with Puerto Ricans having a higher prevalence of childhood asthma (20.7%) than non-Hispanic Blacks (13.4%) or non-Hispanic Whites (7.5%) (2, 3).

Racial or ethnic disparities in asthma are partly mediated by low socioeconomic status (SES) (3–6). Among U.S. residents in 2010, the estimated prevalence of asthma was higher (11.2%) in individuals living below the federal poverty level (FPL) than in those living between 100% and 200% of the FPL (8.7%) or in those living above 200% of the FPL (7.3%)(5). Similar results were reported in 2013, when asthma prevalence was 10.9% among individuals living below the FPL vs. 6% to 7% among those living above the FPL (3). This link between low SES and asthma or asthma morbidity is likely explained by environmental and behavioral risk factors correlated with poverty.

Low SES is associated with poor housing conditions (7) leading to exposure to cockroach allergen, particularly at high levels (8, 9). In one study, residential areas where poverty was

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common (>20% of homes falling below the FPL) had 33 times higher odds of cockroach allergen exposure than other residential areas (10). Not surprisingly, sensitization to cockroach allergen is common among inner-city children (36.8%) (11) and Puerto Rican children (41.5%), who are disproportionately represented among the poor (12).

Cockroach allergen exposure may lead to alterations in adaptive and innate immunity, including allergic sensitization and atopic asthma (13). Cockroach sensitization, a strong risk factor for asthma, occurs more frequently in residents of neighborhoods with high asthma prevalence than in those living in neighborhoods with low asthma prevalence (14). Moreover, cockroach allergen exposure is a causative factor for severe asthma exacerbations, particularly among sensitized individuals (15). For example, children who are exposed and sensitized to cockroach allergen are 3.4 times more likely to be hospitalized for asthma than unexposed children (11). In one neighborhood-based study, housing code violations (which are correlated with cockroach allergen exposure) accounted for 22% of the variability in asthma-related emergency department visits, even after accounting for income (16).

Poverty has been linked to increased circulating IL-6 in children and adults, with limited evidence suggesting a similar link with circulating Th1 or Th2 cytokines in adolescents. In studies of school-aged children and middle-aged adults, low SES during childhood has been associated with increased serum interleukin 6 (IL-6), measured after stimulation of peripheral blood mononuclear cells (PBMCs) with mitogen or endotoxin (17, 18). In a study of 30 adolescents with persistent asthma in Saint Louis (MO), subjects living in low-SES neighborhoods had higher levels of IL-5 and interferon gamma (IFN- γ), measured after mitogen stimulation of PBMCs, than those living in high-SES neighborhoods (19).

Given that cockroach allergen leads to cockroach allergy in humans, surprisingly few studies have examined cockroach allergen exposure and immune responses or circulating cytokines in children. In a study of 560 children with parental history of asthma or allergies, levels of *Blattella germanica* (Bla g 1) in bed dust were not associated with any mononuclear cell cytokine response to adaptive stimuli at birth (20). In another study of 114 children with parental history of asthma or allergies, indoor cockroach allergen level at age 3 months was associated with increased cockroach-specific lymphoproliferative responses at age 2 years (21).

To date, little is known about the relation between poverty or cockroach allergen and adaptive immune responses in school-aged children. We hypothesized that low SES would lead to broadly abnormal adaptive immune responses in school-aged children, and that this would be mainly explained by cockroach allergen exposure. To test this hypothesis, we conducted a cross-sectional study of low household income, cockroach allergen exposure, and plasma cytokine levels (encompassing Th1, Th2, Treg and Th17-related cytokines) among 532 Puerto Rican children living in San Juan (Puerto Rico).

METHODS

Subject Recruitment

Details on subject recruitment and study design have been previously reported (22, 23). In brief, from March 2009 to June 2010, children were recruited from randomly selected households in San Juan, using a multistage probabilistic sampling design. A household was eligible if 1 resident was a child aged 6 to 14 years who had four Puerto Rican grandparents and had lived in the household for at least one year. Of 1,111 eligible households, 438 (~39%) had 1 child with asthma (defined as physician-diagnosed asthma and at least one episode of wheeze in the prior year). From those 438 households, one child with asthma was selected (at random, if there was more than one such child). Similarly, one child without asthma was randomly selected from the remaining 673 households. In an effort to reach a target sample size of ~700 children (which would give us 90% power to detect an OR 2 for exposures with a prevalence 25%), we attempted to enroll a random sample (n=783) of these 1,111 children. Parents of 105 of these 783 eligible households refused to participate or could not be reached. There were no significant differences in age, gender, or area of residence between eligible children who did (n=678 [86.6%]) and did not (n=105 [13.4%]) agree to participate. Of the 678 study participants, 532 (79%) had complete data on household income, cockroach allergen levels in house dust, and plasma cytokines, and were thus included in the current analysis.

Study Procedures

Participants completed a protocol including questionnaires, and collection of house dust and blood samples. The questionnaire, a modified version of one utilized in the Collaborative Study of the Genetics of Asthma (24), was completed by one of the child's parents (usually [93%] the mother). This questionnaire was used to obtain information about the child's general and respiratory health, and socio-demographic characteristics. Dust samples were obtained from three areas in the home: the one in which the child sleeps (usually his/her bedroom), the living room/television room, and the kitchen. The dust was sifted through a 50-mesh metal sieve, and the fine dust was reweighed, extracted, and aliquoted for analysis of allergens from *Blatella germanica* (Bla g 2), *Dermatophagoides pteronyssinus* (Der p 1), cat dander (Feld d 1), dog dander (Can f 1) and mouse urinary protein (Mus m 1), using monoclonal antibody Multiplex array assays with the same reagents employed in the established ELISA (25). Endotoxin was measured in a subset of participants (n=348), using the kinetic chromogenic *Limulus* amoebocyte lysate assay, using multiple dilutions and a 12-point standard curve based on control standard endotoxin from *Escherichia coli*, ranging from 0.0244 to 50.0 EU/ml (26). Non-detectable levels of allergens or endotoxin were assigned a small constant, with all levels then log₁₀-transformed for analysis.

A panel of 14 cytokines (enriched for Th17 cytokines, and including interleukin [IL]-1 β , IL-4, IL-6, IL-10, IL-17A, IL-17F, IL-21, IL-22, IL-23, IL-25, IL-31, IL-33, interferon [IFN]- γ , and tumor necrosis factor [TNF]- α) was measured in plasma samples using the Bio-Plex Pro Human T_H17 cytokine panel on the BioPlex HTF system (Bio-Rad Laboratories Inc., Hercules, CA) as per the manufacturer's instructions, with all samples measured in duplicate. After assigning a small constant (half the lowest detectable level, see

Table 1) to non-detectable levels, plasma cytokine levels were log₁₀-transformed for data analysis, which led to an approximately normal distribution for all cytokines. To account for multiple testing, we calculated a Bonferroni-corrected P value for statistical significance (0.05/14 tests, or 0.00357).

For each participant, global percentage of African ancestry was estimated from genome-wide genotypic data, using the Local Ancestry in adMixed Populations (LAMP) method and software (27, 28), as previously described (22). IgE to cockroach allergen (Bla g 2) was measured in plasma using the UniCap 100 system (Kalamazoo, MI). A positive cockroach-specific IgE was defined as ≥ 0.35 IU/ml.

Written parental consent was obtained for participating children, from whom written assent was also obtained. The study was approved by the Institutional Review Boards of the University of Puerto Rico (San Juan, Puerto Rico), Brigham and Women's Hospital (Boston, Massachusetts), and University of Pittsburgh (Pittsburgh, Pennsylvania).

Statistical Analysis

Our outcome of interest was log-10 transformed plasma cytokine levels. Our exposures of interest were low household income (defined as <\$15,000/year, the median household income for Puerto Rico in 2008–2009)(29) and log-transformed cockroach allergen level in house dust. Low household income was selected as our primary indicator of SES on the basis of our prior work in Puerto Rico. Linear regression was used for bivariate and multivariable analyses of plasma cytokines. All multivariable models of low income or cockroach allergen (first analyzed separately, and then within the same model) and plasma cytokines were adjusted for age, gender and case-control status. Because of their potential correlation with household income and/or cockroach allergen, the following covariates were examined in bivariate analyses and also considered for inclusion in the multivariable models: low parental education (neither parent completed high school vs. at least one parent completed high school), percentage of African ancestry (22), current exposure to second-hand smoke (SHS)(30), early-life (in utero or before age 2 years) exposure to SHS, log-transformed levels of other allergens (dust mite, dog dander, cat dander and mouse urinary protein) and endotoxin (31, 32), and residential distance to a major roadway (in meters)(33). In secondary analyses, we stratified children by case-control status or by having a positive cockroach-specific IgE; these secondary analyses were not adjusted for endotoxin levels due to small sample size.

All statistical analyses were performed using SAS statistical software, version 9.4 (SAS Institute; Cary, NC).

RESULTS

Compared with subjects excluded from this analysis (n=146), those included (n=532) were significantly more likely to have low income and low parental education. However, there were no significant differences in indoor level of any allergen (including cockroach) or endotoxin, age, gender, early or current exposure to SHS, residential distance to a major

road or percentage of African ancestry between those who were and were not included in the analysis (data not shown).

Table 2 shows the main characteristics of the 532 study participants. Compared with control subjects, children with asthma (cases) were significantly younger, and more likely to be male and to have been exposed to a lower cat allergen level. There were no significant differences in household income, parental education, exposure to SHS (either currently or in early-life), residential distance to a major road, percentage of African ancestry, or house dust levels of other allergens (Bla g 2, Der p 1, Can f 1 or Mus m 1) or endotoxin between cases and control subjects.

In bivariate analyses, cockroach allergen was most significantly and consistently associated with plasma cytokines (Table 3). Cockroach allergen was significantly associated with decreased IL-17A, as well as increased IL-4, IL-6, IL-10, IL-17F, IL-21, IL-22, IL-25, IL-31, IL-33, IFN- γ , and TNF- α . Cat and dog allergen levels were each significantly associated with increased IL-10 and IL-22. Age, gender, low household income, low parental education, and residential distance to a road were each significantly associated with 1–2 cytokines. None of the other variables (dust mite allergen, mouse allergen, endotoxin, early SHS, current SHS, and African ancestry) was significantly associated with any cytokine.

Figure 1 and Table e1 show the multivariable analysis of low income and/or cockroach allergen and plasma cytokines. After adjustment for age, gender and case-control status, low income was not significantly associated with any cytokine (Model A in Table e1). In an analysis adjusting for the same covariates as in Model A (minus income), cockroach allergen was significantly associated with 12 of the 14 cytokines tested (Model B in Table e1). We then conducted a multivariable analysis including both low household income and cockroach allergen, plus levels of cat, dog, and mouse allergens (Figure 1 and Model C in Table e1). In this analysis, cockroach allergen was significantly associated with decreased IL-17A, as well as with increased IL-4, IL-10, IL-17F, IL-21, IL-25, IL-31, IFN- γ and TNF- α . As an example, each log₁₀-unit increment in cockroach allergen level was significantly associated with an increment of 1.71 pg/ml in IL-4 level (95% CI=1.28 to 2.29 pg/ml). We then repeated the analysis after additional adjustment for house dust endotoxin (Model D in Table e1), obtaining similar results despite smaller sample size.

Tables 2 and 3 show the results of the multivariable analysis after stratification by case-control status. Among cases, this analysis yielded similar results to those of the analysis in all children. Although most of the associations observed among all 532 children (except for that for IL-10) became non-statistically significant in the 272 cases, there was little or no change in the magnitude and direction of the estimated effect of low income or cockroach allergen on plasma cytokines (eTable 2). Similar results were obtained in the multivariable analysis among 260 control subjects, in whom the associations observed among all 532 children were minimally changed with regard to direction or magnitude (eTable 3). Among controls, cockroach allergen remained significantly associated with decreased IL-17A, as well as with increased IL-4, IL-17F, IL-21, IL-25, and IFN- γ .

We then repeated the multivariable analysis in all participants after stratification by cockroach allergy (i.e. a positive IgE to cockroach). Among children with cockroach allergy, this analysis yielded similar results to those of the analysis in all children (Table 4). In particular, cockroach allergen remained significantly associated with decreased IL-17A, as well as with increased IL-4, IL-10, IL-17F, IL-21, IL-25, and IFN- γ . Among children without cockroach allergy, the associations observed among all 532 children remained in the same direction but were weaker. In these children, cockroach allergen was not significantly associated with any cytokine level (data not shown).

DISCUSSION

In our study, low household income was not significantly associated with any plasma cytokine. On the other hand, cockroach allergen was significantly associated with decreased IL-17A and increased levels of eight cytokines (IL-4, IL-10, IL-17F, IL-21, IL-25, IL-31, IFN- γ and TNF- α), independently of household income and other allergens. Most associations observed in all children remained similar with regard to magnitude, direction, and statistical significance among children with cockroach allergy, but became weaker and non-statistically significant among children without cockroach allergy. Our results thus suggest that cockroach allergen has broad effects on Th1, Th2, Th17 and Treg immune responses in school-aged children living in a tropical environment (Puerto Rico), particularly among children sensitized to cockroach allergen.

Previous studies in children or adolescents have shown an association between low SES and serum IL-5, IL-6 and IFN- γ (17, 34), but we did not replicate those findings for IL-6 or IFN- γ . Our findings are partly consistent with those of an experimental model in which exposure to German cockroach feces was associated with increased production of IL-6, IL-23 and IL-12p70 from bone marrow-derived myeloid dendritic cells (13). In that mouse model, levels of IL-5, IL-17A and IL-6 in whole lung cultures were increased 18 hours after exposure to cockroach allergen feces, suggesting a mixed Th2 and Th17 response (13). Our results are further supported by other experiments showing that cockroach allergen can act through PAR-2 pathways to induce innate cellular immunity (15), as well as by known effects of cockroach allergen on Th2 (allergic) immune responses.

Our study has several strengths, including a well-characterized cohort of high-risk minority children and the ability to adjust for confounding factors. We also recognize several study limitations. First, we cannot assess temporal relationships between low SES or cockroach allergen and plasma cytokines in a cross-sectional study. For example, the observed association between cockroach allergen and IL-10 may represent an attempt to down-regulate Th2 immune responses in exposed children. However, cockroach allergen has previously been proven as a causal factor for asthma morbidity in longitudinal studies and clinical trials (15). Second, selection bias is possible in any observational study. Nonetheless, cockroach allergen level was similar and not significantly different between children who were and were not included in this analysis, making selection bias unlikely as a major explanation for our results. Third, our findings are not generalizable to children living in temperate climates (or those living in households with no or low levels of cockroach allergen), yet relevant to children living in inner-city areas in the U.S. Fourth, we

had limited statistical power to examine modification of the effect of cockroach allergen by case-control status or cockroach allergy. However, we found similar results in cases and control subjects, and were able to demonstrate differences with regard to the strength and significance of the associations between children with and without cockroach allergy. Fifth, as in prior work, we used one measure (low household income) as our primary indicator of SES, but we obtained the same results for cockroach allergen in multivariable analyses including an alternative measure of SES (low parental education). Finally, we did not examine exposure to violence or other indicators of psychosocial stress (which are correlated with low SES and may affect immune responses)(35), since we only had such data in a subset of participants.

In summary, our findings suggest that indoor exposure to cockroach allergen leads to strong stimulation of adaptive immune responses (including Th1, Th2, Treg and Th17 responses) in Puerto Rican children, particularly among those sensitized to cockroach. Thus, cockroach abatement (through extermination or improved housing conditions)(36) may help reduce the burden of allergic diseases and asthma in these children.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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References

1. WHO. Asthma. 2013. Available from: <http://www.who.int/mediacentre/factsheets/fs307/en/>. (accessed on August 12, 2016)
2. Rosser FJ, Forno E, Cooper PJ, Celedón JC. Asthma in hispanics. An 8-year update. *Am J Respir Crit Care Med*. 2014; 189:1316–1327. [PubMed: 24881937]
3. Prevention CfDCA. Most recent asthma data. 2013. Oct 2. 2015 [accessed on August 12, 2016] Available from: http://www.cdc.gov/asthma/most_recent_data.htm
4. Grant EN, Lyttle CS, Weiss KB. The relation of socioeconomic factors and racial/ethnic differences in us asthma mortality. *Am J Public Health*. 2000; 90:1923–1925. [PubMed: 11111268]
5. Akinbami L, Moorman J, Bailey C, et al. Trends in asthma prevalence, health care use, and mortality in the United States, 2001–2010. *NCHS Data Brief*. 2012; 94
6. Litonjua AA, Carey VJ, Weiss ST, Gold DR. Race, socioeconomic factors, and area of residence are associated with asthma prevalence. *Pediatr Pulmonol*. 1999; 28:394–401. [PubMed: 10587412]
7. Pacheco CM, Ciaccio CE, Nazir N, et al. Homes of low-income minority families with asthmatic children have increased condition issues. *Allergy Asthma Proc*. 2014; 35:467–474. [PubMed: 25584914]
8. Cohn RD, Arbes SJ, Jaramillo R, Reid LH, Zeldin DC. National prevalence and exposure risk for cockroach allergen in U.S. Households. *Environ Health Perspect*. 2006; 114:522–526. [PubMed: 16581539]

9. Sarpong SB, Hamilton RG, Eggleston PA, Adkinson NF Jr. Socioeconomic status and race as risk factors for cockroach allergen exposure and sensitization in children with asthma. *J Allergy Clin Immunol*. 1996; 97:1393–1401. [PubMed: 8648037]
10. Kitch BT, Chew G, Burge HA, et al. Socioeconomic predictors of high allergen levels in homes in the greater boston area. *Environ Health Perspect*. 2000; 108:301–307. [PubMed: 10753087]
11. Rosenstreich DL, Eggleston P, Kattan M, et al. The role of cockroach allergy and exposure to cockroach allergen in causing morbidity among inner-city children with asthma. *N Engl J Med*. 1997; 336:1356–1363. [PubMed: 9134876]
12. Montealegre F, Meyer B, Chardon D, et al. Comparative prevalence of sensitization to common animal, plant and mould allergens in subjects with asthma, or atopic dermatitis and/or allergic rhinitis living in a tropical environment. *Clin Exp Allergy*. 2004; 34:51–58. [PubMed: 14720262]
13. Page K, Zhou P, Ledford JR, et al. Early immunological response to german cockroach frass exposure induces a th2/th17 environment. *J Innate Immun*. 2011; 3:167–179. [PubMed: 21051864]
14. Olmedo O, Goldstein IF, Acosta L, et al. Neighborhood differences in exposure and sensitization to cockroach, mouse, dust mite, cat, and dog allergens in new york city. *J Allergy Clin Immunol*. 2011; 128:284–292.e287. [PubMed: 21536321]
15. Kanchongkittiphon W, Mendell MJ, Gaffin JM, Wang G, Phipatanakul W. Indoor environmental exposures and exacerbation of asthma: An update to the 2000 review by the Institute of Medicine. *Environ Health Perspect*. 2015; 123:6–20. [PubMed: 25303775]
16. Beck AF, Huang B, Chundur R, Kahn RS. Housing code violation density associated with emergency department and hospital use by children with asthma. *Health Aff (Millwood)*. 2014; 33:1993–2002. [PubMed: 25367995]
17. Azad MB, Lissitsyn Y, Miller GE, Becker AB, HayGlass KT, Kozyrskyj AL. Influence of socioeconomic status trajectories on innate immune responsiveness in children. *PLoS One*. 2012; 7:e38669. [PubMed: 22685596]
18. Carroll JE, Cohen S, Marsland AL. Early childhood socioeconomic status is associated with circulating interleukin-6 among mid-life adults. *Brain Behav Immun*. 2011; 25:1468–1474. [PubMed: 21672624]
19. Chen E, Strunk RC, Trethewey A, Schreier HM, Maharaj N, Miller GE. Resilience in low-socioeconomic-status children with asthma: Adaptations to stress. *J Allergy Clin Immunol*. 2011; 128:970–976. [PubMed: 21824649]
20. Wood RA, Bloomberg GR, Kattan M, et al. Relationships among environmental exposures, cord blood cytokine responses, allergy, and wheeze at 1 year of age in an inner-city birth cohort (urban environment and childhood asthma study). *J Allergy Clin Immunol*. 2011; 127:913–919. e911–916. [PubMed: 21333343]
21. Finn PW, Boudreau JO, He H, et al. Children at risk for asthma: Home allergen levels, lymphocyte proliferation, and wheeze. *J Allergy Clin Immunol*. 2000; 105:933–942. [PubMed: 10808174]
22. Brehm JM, Acosta-Perez E, Klei L, et al. African ancestry and lung function in puerto rican children. *J Allergy Clin Immunol*. 2012; 129:1484–1490.e1486. [PubMed: 22560959]
23. Rosas-Salazar C, Ramratnam SK, Brehm JM, et al. Prematurity, atopy, and childhood asthma in puerto ricans. *J Allergy Clin Immunol*. 2014; 133:357–362. [PubMed: 24139607]
24. Blumenthal MN, Banks-Schlegel S, Bleecker ER, Marsh DG, Ober C. Collaborative studies on the genetics of asthma-National Heart, Lung and Blood Institute. *Clin Exp Allergy*. 1995; 25
25. Luczynska CM, Arruda LK, Platts-Mills TA, Miller JD, Lopez M, Chapman MD. A two-site monoclonal antibody elisa for the quantification of the major dermatophagoides spp. Allergens, der p i and der f i. *J Immun Methods*. 1989; 118:227–235.
26. Thorne PS. Inhalation toxicology models of endotoxin- and bioaerosolinduced inflammation. *Toxicology*. 2000; 152:13–23. [PubMed: 11090935]
27. Sankararaman S, Sridhar S, Kimmel G, Halperin E. Estimating local ancestry in admixed populations. *Am J Hum Genet*. 2008; 82:290–303. [PubMed: 18252211]
28. Pasaniuc B, Sankararaman S, Kimmel G, Halperin E. Inference of locus-specific ancestry in closely related populations. *Bioinformatics*. 2009; 25:i213–221. [PubMed: 19477991]
29. Household income for states: 2008 and 2009. Available from: <http://www.census.gov/prod/2010pubs/acsbr09-2.pdf> (accessed on October 5, 2011)

30. Ciaccio CE, DiDonna A, Kennedy K, et al. Secondhand tobacco smoke exposure in low-income children and its association with asthma. *Allergy Asthma Proc.* 2014; 35:462–466. [PubMed: 25584913]
31. Thorne PS, Mendy A, Metwali N, et al. Endotoxin exposure: Predictors and prevalence of associated asthma outcomes in the united states. *Am J Respir Crit Care Med.* 2015; 192:1287–1297. [PubMed: 26258643]
32. Celedón JC, Milton DK, Ramsey CD, et al. Exposure to dust mite allergen and endotoxin in early life and asthma and atopy in childhood. *J Allergy Clin Immunol.* 2007; 120:144–149. [PubMed: 17507083]
33. Rosser F, Brehm JM, Forno E, et al. Proximity to a major road, Vitamin D insufficiency, and severe asthma exacerbations in Puerto Rican children. *Am J Respir Crit Care Med.* 2014; 190:1190–1193. [PubMed: 25398111]
34. Chen E, Hanson MD, Paterson LQ, Griffin MJ, Walker HA, Miller GE. Socioeconomic status and inflammatory processes in childhood asthma: The role of psychological stress. *J Allergy Clin Immunol.* 2006; 117:1014–1020. [PubMed: 16675327]
35. Rosenberg SL, Miller GE, Brehm JM, et al. Stress and asthma: Novel insights on genetic, epigenetic, and immunologic mechanisms. *J Allergy Clin Immunol.* 2014; 134:1009–1015. [PubMed: 25129683]
36. Portnoy J, Chew GL, Phipatanakul W, et al. Joint Task Force on Practice P. Environmental assessment and exposure reduction of cockroaches: A practice parameter. *J Allergy Clin Immunol.* 2013; 132:802–808. e801–825. [PubMed: 23938214]

Figure 1. Multivariable analysis of cockroach allergen exposure and plasma cytokines in study participants

*Bla g 2 level in house dust (U/g) and plasma cytokine levels (pg/ml) were log-transformed for data analysis.

For each cytokine, models were adjusted for age, gender, low household income, case-control status, and house dust levels of: Mus m 1, Fel d 1, and Can f 1. All reported associations were significant at $P < 0.00357$ (0.05/14 tests).

Table 1

Lowest detectable limit of assays used for cytokine measurements (n=532)

Cytokine	Lowest limit of detection (LLD, pg/ml)	Number (%) with value under LLD
Interleukin (IL)-1 β	0.02	19 (3.6)
IL-4	0.52	210 (39.5)
IL-6	0.67	56 (10.5)
IL-10	0.3	166 (31.2)
IL-17A	0.49	142 (26.7)
IL-17F	0.8	197 (37.0)
IL-21	2.13	330 (62.0)
IL-22	0.03	177 (33.3)
IL-23	1.55	474 (89.1)
IL-25	0.07	3 (0.56)
IL-31	0.49	97 (18.2)
IL-33	0.58	39 (7.33)
Interferon gamma (IFN- γ)	0.43	175 (32.9)
Tumor necrosis factor alpha (TNF- α)	0.07	1 (0.2)

Table 2

Main characteristics of study participants

	<i>Controls (n=260)</i>	<i>Cases (n=272)</i>
Age (years) *	11.1 (2.7)	10.6 (2.6)†
Male gender	125 (48.1 %)	156 (57.4 %) ‡
Annual household income <\$15,000	171 (65.8 %)	188 (69.1 %)
Neither parent completed high school	57 (21.9%)	51 (18.8%)
Bl a g 2 in house dust (U/g)‡	0.272 (0.667)	0.311 (0.721)
Der p 1 in house dust (µg/g)‡	0.648 (0.504)	0.663 (0.513)
Mus m 1 in house dust (ng/g)‡	0.957 (0.930)	1.040 (1.018)
Fel d 1 in house dust (mcg/g)‡	−1.344 (1.100)	−1.558 (0.913) ‡
Can f 1 in house dust (mcg/g)‡	−0.713 (0.885)	−0.708 (0.981)
Endotoxin (EU/ml)‡§	3.20 (0.38)	3.28 (0.41)
Second-hand smoke in early life (in utero or before age 2 years)	109 (42.1 %)	135 (49.6%)
Current second-hand smoke	97 (37.3 %)	118 (43.4%)
Residential distance to a major road (meters)	376 (301)	338 (270)
African ancestry (percent)	25.2 (12.9)	25.4 (11.9)

* Data are presented as mean (SDs) for continuous variables and number (percentage) for binary variables

† P-value <0.05 for comparison between groups

‡ Levels were log10-transformed for data analysis

§ Based on data for 348 children (174 cases and 174 control subjects)

Table 3

Bivariate linear regression analyses of plasma cytokines[§] in 532 participating children

	IL-1	IL-4	IL-6	IL-10	IL-17A	IL-17F	IL-21	IL-22	IL-23	IL-25	IL-31	IL-33	IFN- γ	TNF- α
Age (years)	-0.006	-0.014	-0.027 [‡]	-0.017	-0.019	-0.037	0.006	-0.020	-0.013	-0.014	-0.028	-0.005	-0.046	-0.013 [‡]
Male Gender	0.019	-0.018	0.100	0.150	-0.007	0.018	0.069	0.157	0.033	0.101	0.186	0.062	0.154	0.064 [‡]
Low household income [¶]	0.005	0.217	0.101	0.322 [‡]	-0.204	0.263	0.070	0.077	-0.038	0.085	0.240	0.005	0.148	0.056
Low parental education [¶]	0.081	0.106	0.155	0.312	-0.216	0.152	0.122	0.232	-0.068	0.109	0.205	0.162	0.288	0.075 [‡]
Bla g 2 (U/g)	0.057	0.289 [‡]	0.106 [‡]	0.341 [‡]	-0.231 [‡]	0.290 [‡]	0.181 [‡]	0.180 [‡]	-0.054	0.103 [‡]	0.256 [‡]	0.154 [‡]	0.291 [‡]	0.055 [‡]
Der p 1 (μ g/g)	0.055	-0.016	0.053	0.124	-0.099	0.059	0.051	0.046	0.018	0.043	0.130	0.052	0.055	0.012
Mus m 1 (mg/g)	-0.013	-0.138	-0.022	-0.104	0.030	-0.103	-0.066	-0.076	0.012	-0.022	-0.058	-0.008	-0.086	-0.013
Fel d 1 (mcg/g)	0.024	0.088	0.010	0.153 [‡]	-0.101	0.013	0.073	0.145 [‡]	0.011	0.038	0.084	0.065	0.015	0.015
Can f 1 (mcg/g)	0.015	0.112	0.046	0.144 [‡]	0.011	0.028	0.049	0.131 [‡]	0.032	0.031	0.089	0.063	0.025	0.014
Endotoxin (EU/ml)	0.024	0.058	0.019	0.128	-0.140	0.165	0.079	0.055	0.058	0.052	-0.007	-0.012	0.069	0.051
Early-life SHS *	0.056	-0.005	0.042	0.138	0.013	0.110	0.102	0.100	-0.020	0.005	0.072	0.049	0.061	0.052
Current SHS *	-0.012	-0.038	0.011	-0.007	-0.052	0.113	-0.003	-0.093	-0.115	0.013	0.044	-0.043	0.035	0.003
Residential distance to a major road **	-0.010	-0.030	-0.02	-0.03	0.003	-0.04	-0.02	-0.002	0.01	-0.01	-0.05 [‡]	-0.01	-0.04	-0.005
African ancestry	-0.0007	0.002	0.0003	0.004	-0.0009	0.0006	-0.0012	0.0039	0.0026	0.0009	0.0010	0.0024	0.0043	-0.0009

[§] Plasma cytokine levels and allergen levels were log-transformed for data analysis

[¶] Low household income= <\$15,000 per year, low parental education=neither parent completed high school

* SHS: Second-hand smoke. Early-life SHS: in utero or before age 2 years.

** Per every 100 meters

[‡] P < 0.00357 (0.05/14)

Endotoxin levels available in 348 of the 532 study participants

Table 4

Multivariable analysis of cockroach allergen exposure and plasma cytokines* in children with a positive IgE to cockroach

Cytokine	Covariate	Model A (n=244)	Model B (n=244)	Model C (n=244)
IL-1	Low income [†]	−0.287 (−0.188, 0.131)	–	−0.057 (−0.218, 0.105)
	Bla g 2	–	0.104 (0.001, 0.208)	0.090 (−0.022, 0.201)
IL-4	Low income	0.089 (−0.194, 0.373)	–	0.032 (−0.244, 0.309)
	Bla g 2	–	0.372 [‡] (0.193, 0.551)	0.311 [‡] (0.120, 0.502)
IL-6	Low income	0.092 (−0.062, 0.245)	–	0.063 (−0.090, 0.215)
	Bla g 2	–	0.183 [‡] (0.086, 0.281)	0.164 (0.059, 0.269)
IL-10	Low income	0.358 (0.045, 0.668)	–	0.295 (−0.005, 0.596)
	Bla g 2	–	0.481 [‡] (0.286, 0.676)	0.398 [‡] (0.190, 0.605)
IL-17A	Low income	−0.372 (−0.617, −0.128)	–	−0.321 (−0.565, −0.077)
	Bla g 2	–	−0.218 (−0.378, −0.058)	−0.235 [‡] (−0.403, −0.066)
IL-17F	Low income	0.258 (−0.046, 0.562)	–	0.199 (−0.103, 0.501)
	Bla g 2	–	0.382 [‡] (0.188, 0.576)	0.364 [‡] (0.155, 0.573)
IL-21	Low income	0.015 (−0.210, 0.239)	–	−0.055 (−0.276, 0.165)
	Bla g 2	–	0.322 [‡] (0.182, 0.463)	0.316 [‡] (0.164, 0.468)
IL-22	Low income	0.056 (−0.220, 0.332)	–	0.009 (−0.262, 0.280)
	Bla g 2	–	0.273 [‡] (0.097, 0.450)	0.178 (−0.009, 0.365)
IL-23	Low income	−0.082 (−0.220, 0.055)	–	−0.078 (−0.217, 0.063)
	Bla g 2	–	−0.052 (−0.142, 0.037)	−0.063 (−0.159, 0.034)
IL-25	Low income	0.119 (−0.003, 0.241)	–	0.090 (−0.030, 0.210)
	Bla g 2	–	0.169 [‡] (0.092, 0.246)	0.148 [‡] (0.065, 0.231)
IL-31	Low income	0.276 (0.008, 0.544)	–	0.219 (−0.043, 0.480)
	Bla g 2	–	0.378 [‡] (0.208, 0.548)	0.292 [‡] (0.111, 0.473)
IL-33	Low income	0.006 (−0.200, 0.212)	–	−0.056 (−0.260, 0.148)
	Bla g 2	–	0.246 [‡] (0.115, 0.377)	0.221 [‡] (0.080, 0.361)
IFN-γ	Low income	0.164 (−0.157, 0.485)	–	0.099 (−0.219, 0.418)
	Bla g 2	–	0.408 [‡] (0.204, 0.612)	0.374 [‡] (0.154, 0.593)
TNF-α	Low income	0.052 (−0.026, 0.130)	–	0.036 (−0.042, 0.114)
	Bla g 2	–	0.090 [‡] (0.041, 0.140)	0.078 (0.024, 0.131)

* Plasma cytokine levels and allergen levels were log10-transformed for data analysis

[†] Low income defined as an annual household income lower than \$15,000 per year

[‡] P < 0.00357 (0.05/14 tests)

All models (A to D) were adjusted for age, gender and case-control status. Model A included low income, Model B included cockroach allergen (Bla g 2) level in house dust, and Model C included both low income and Bla g 2 house dust level, plus house dust levels of Mus m 1, Fel d 1, and Can f 1. Model D included all variables in Model C, plus endotoxin levels.