Early-life cockroach allergen and polycyclic aromatic hydrocarbon exposures predict cockroach sensitization among inner-city children

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Background: Sensitization to cockroach is one of the strongest identified risk factors for greater asthma morbidity in low-income urban communities; however, the timing of exposures relevant to the development of sensitization has not been elucidated fully. Furthermore, exposure to combustion byproducts, including polycyclic aromatic hydrocarbons (PAHs), can augment the development of allergic sensitization.
Objective: We sought to test the hypotheses that domestic cockroach allergen measured prenatally would predict cockroach sensitization in early childhood and that this association would be greater for children exposed to higher PAH concentrations.
Methods: Dominican and African American pregnant women living in New York City were enrolled. In the third trimester expectant mothers wore personal air samplers for measurement of 8 nonvolatile PAHs and the semivolatile PAH pyrene, and dust was collected from homes for allergen measurement. Glutathione-S-transferase μ 1 *(GSTM1)* gene polymorphisms were measured in children. Allergen-specific IgE levels were measured from the children at ages 2, 3, 5, and 7 years.
Results: Bla g 2 in prenatal kitchen dust predicted cockroach sensitization at the ages of 5 to 7 years (adjusted relative risk [RR], 1.15; $P = .001$; n = 349). The association was observed only among children with greater than (RR, 1.22; $P = .001$) but not less than (RR, 1.07; $P = .24$) the median sum of 8 nonvolatile PAH levels. The association was most pronounced among children with higher PAH levels and null for the *GSTM1* gene (RR, 1.54; $P = .001$).
Conclusions: Prenatal exposure to cockroach allergen was associated with a greater risk of allergic sensitization. This risk was increased by exposure to nonvolatile PAHs, with children null for the *GSTM1* mutation particularly vulnerable. (J Allergy Clin Immunol 2013;131:886-93.)

Key words: *Bla g 2, cockroach, polycyclic aromatic hydrocarbon, IgE, allergy, inner-city, GSTM, GSTP*

Sensitization to cockroach allergens is one of the strongest identified risks for greater asthma morbidity in low-income urban populations.[1] Although several studies among preschool- and school-age children have demonstrated associations between cockroach allergen measured in the home and sensitization,[2,3] studies have not yet demonstrated prospectively whether cockroach allergen in a child's house dust predicts the development of sensitization. Understanding whether exposure to cockroach allergen in the prenatal and early postnatal time period leads to sensitization could inform primary prevention interventions.

Emerging evidence suggests that environmental coexposures in addition to allergens, including microbial products and diesel exhaust particles (DEPs), could modify the risk of allergic sensitization.[4,5] For example, Diaz-Sanchez et al[6,7] demonstrated in human *in vivo* experiments that DEPs can act as adjuvants to promote sensitization to novel allergens and enhance the T_H2 response to established sensitization. Like cockroach allergen, DEPs and other combustion products are ubiquitous exposures in urban communities, beginning prenatally.[8-10] One class of combustion byproducts found in DEPs, the polycyclic aromatic hydrocarbon (PAH), has been investigated extensively for its contribution to the development of cancer.[11,12] We and others also demonstrated associations between PAHs and respiratory outcomes, and these associations appeared to differ with exposure to a composite variable of 8 highly correlated nonvolatile PAHs ($\sum_8 PAH_{nonvolatile}$) compared with exposure to the less well-correlated and semivolatile pyrene.[13-15]

Common polymorphisms in glutathione-S-transferase μ (GSTM) and glutathione-S-transferase π *(GSTP)* have been shown

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Abbreviations used
DEP: Diesel exhaust particle
GSTM: Glutathione-S-transferase μ gene
GSTP: Glutathione-S-transferase π gene
KLH: Keyhole limpet hemocyanin
PAH: Polycyclic aromatic hydrocarbon
$\sum_8 PAH_{nonvolatile}$: Sum of 8 nonvolatile polycyclic aromatic hydrocarbons
RR: Relative risk

to alter the conjugation kinetics of PAHs.[16-18] These same polymorphisms appear to modify the adjuvant effect of DEPs on allergic sensitization[19] and have been associated with other allergic disease–related health outcomes (eg, lung function and asthma).[20-23] However, whether these polymorphisms modify the effect of PAHs on allergic sensitization still needs to be elucidated.

We hypothesized that domestic cockroach allergen measured prenatally would predict cockroach sensitization in early childhood and that the magnitude of this association would be greater for children also exposed prenatally to higher PAH levels. We tested this hypothesis in a well-characterized birth cohort study of children of women of African American and Dominican ethnicity living in low-income neighborhoods in New York City. We also tested whether the magnitude of the association between Bla g 2, PAHs, and sensitization would be greater among children with polymorphisms in *GSTM* and *GSTP*.

METHODS

As part of the Columbia Center for Children's Environmental Health, 727 nonsmoking, pregnant African American or Dominican women between the ages of 18 and 35 years who were living in Northern Manhattan and the South Bronx were enrolled.[8,10,24] Women with a history of asthma or allergies were not recruited preferentially. Detailed questionnaires were administered to the participants before the child was born. Environmental tobacco smoke exposure during pregnancy was assessed by means of questionnaire. The children were followed prospectively (Fig 1). Columbia University's Institutional Review Board approved this study.

Allergen exposures

Bed and kitchen dust samples were collected from the participants' homes during the third trimester and again when the child was 1, 3, and 5 years old.[24] Dust samples were extracted without prior sieving, but large debris was removed. Kitchen and bed samples were assayed for Bla g 2 (Indoor Biotechnologies, Charlottesville, Va) and mouse urinary protein, and bed samples were assayed also for Der f 1 by using an ELISA, as previously described.[24] Estimations of Bla g 2 and Der f 1 concentrations were based on the universal allergen standard curve, and thus comparisons with our results published before 2011 and those of other studies not using the universal allergen standard will require conversions, as previously described.[3,25] For results of less than the limit of detection, values of half of the limit of detection were used in analyses.

PAH measures

Personal air sampling was conducted for the mothers during the third trimester of pregnancy, as previously described.[10,13] Briefly, women wore a small backpack during the waking hours and placed the sampler near the bed at night. Air was sampled for 48 hours at 4 L/min. Particulate matter of 2.5 μm in diameter or less was collected on a quartz microfiber filter, and semivolatile components were collected on polyurethane foam plug backups. Filters and polyurethane foams were extracted together and analyzed by using gas chromatography/mass spectrometry at the Southwest Research Institute for benz(a)anthracene, benzo(b)fluoranthene, benzo(k)fluoranthene, benzo(ghi)perylene, benzo(a)pyrene, chrysene/isochrysene, dibenz(a,h)anthracene, indeno(1,2,3-cd)pyrene, and pyrene.[26]

IgE

IgE antibodies to cockroach, mouse, *Dermatophagoides farinae*, and cat were measured in sera collected from children at ages 2, 3, 5, and 7 years. As described previously, 40 of the initial samples collected at ages 2 and 3 years were assayed by using the Fluorescence Allergosorbent Test (Bio-Whitaker, Walkersville, Md), and all of the subsequent samples were assayed by using ImmunoCAP (ThermoFisher, Uppsala, Sweden).[27,28] A subset of samples was analyzed by using both methods to confirm concordance (data not shown). Specific IgE levels of greater than 0.35 IU/mL were considered positive. Children with at least 1 positive specific IgE level at ages 5 or 7 years were considered positive at ages 5 to 7 years, and a similar variable was created for ages 2 to 3 years.

GSTM and *GSTP* genotyping

DNA was isolated from the child's cord blood cells or at a later age (between the ages of 2 and 5 years) from PBMCs. The deletion of *GSTM1* was assessed by using the Multiplex PCR system.[29] Children who were homozygous (+/+) or heterozygous (−/+) for *GSTM1* were classified as *GSTM1* positive, and those who were homozygous deleted (−/−) were classified as *GSTM1* null. Polymorphisms in *GSTP1* (Ile105Val, rs1695) were assessed with the use of the ABI 7500 System in the TaqMan genotyping assay with primers and probes obtained from Applied Biosystems (Foster City, Calif). GST-P1 primers were GST-P1-F CCTGGTGGACATGGTGAATGAC and GST-P1-R CAGATGCTCACATAGTTGGTGTAGA.

Data analyses and statistics

Analyses were restricted to children who had complete data on PAH and Bla g 2 levels measured prenatally and IgE levels measured at age 5 years, 7 years, or both. The greatest Bla g 2 concentration measured in kitchen dust at age 1, 3, or 5 years was assigned as the postnatal Bla g 2 level. Allergen and PAH levels were approximately natural log-normally distributed; natural log-transformed variables were used in analyses. For visualization of the associations between allergen exposure and allergic sensitization, logistic regression curves were plotted. For multivariable analyses of these associations, relative risks (RRs) with 95% CIs were calculated by using binomial regressions in generalized estimating equations. The potential cofounders and covariates of sex, race/ethnicity, maternal asthma, material hardship, prenatal environmental tobacco smoke exposure, birth order, and age of the child at IgE measurement were included in all multivariable models. Multiplicative interaction terms were included in the multivariable models to test for effect modification. As we have reported previously, the 8 nonvolatile PAHs showed a high degree of correlation with each other, but the semivolatile pyrene did not correlate well with these other PAHs.[13] Therefore in keeping with the previous analytic strategy, PAH levels were analyzed as the sum of 8 individual nonvolatile PAHs ($\sum_8 PAH_{nonvolatile}$) and, separately, pyrene. A previously defined home heating season variable was included in all models that included PAHs or pyrene.[26] Data were analyzed in SPSS software (version 17; SPSS, Chicago, Ill) and visualized in R version 2.14.0 software.

RESULTS

There were 349 children with prenatal Bla g 2, prenatal PAH, and IgE levels measured at least once between the ages of 5 and 7 years (see Fig E1 in this article's Online Repository at www.jacionline.org). Of these, 317 children also had a Bla g 2 level measured at least once in kitchen dust between the ages of 1 and 5 years. The demographics of the mothers are reported in Table I.[30] When comparing children who were included in the analyses with those who were enrolled but excluded from the