Ann Allergy Asthma Immunol 114 (2015) 193–198

Contents lists available at ScienceDirect

# Early-life mold and tree sensitivity is associated with allergic eosinophilic rhinitis at 4 years of age

Christopher D. Codispoti, MD, PhD [*,†,§]; David I. Bernstein, MD [*]; Linda Levin, PhD [†]; Tiina Reponen, PhD [†]; Patrick H. Ryan, PhD [†,‡]; Jocelyn M. Biagini Myers, PhD [§]; Manuel Villareal, MD [*]; Jeff Burkle, BS [†]; Zana Lummus, PhD [*]; James E. Lockey, MD, MS [*,†]; Gurjit K. Khurana Hershey, MD, PhD [‡]; and Grace K. LeMasters, PhD [†]

[*] Department of Internal Medicine, Divisions of Immunology/Allergy and Pulmonary Medicine, University of Cincinnati, Cincinnati, Ohio
[†] Department of Environmental Health, University of Cincinnati, Cincinnati, Ohio
[‡] Division of Asthma Research, Children's Hospital Medical Center, Rush University Medical Center, Chicago, Illinois
[§] Division of Immunology and Microbiology, Rush University Medical Center, Chicago, Illinois

ARTICLE INFO

*Article history:*
Received for publication June 26, 2014.
Received in revised form December 10, 2014.
Accepted for publication December 12, 2014.

ABSTRACT

**Background:** Nasal eosinophils are a biomarker for allergic rhinitis (AR) and are associated with increased symptom severity.
**Objective:** To identify predictors of allergic eosinophilic rhinitis (AER) in early childhood in children at higher risk for chronic allergic respiratory disorders.
**Methods:** In the Cincinnati Childhood Allergy and Air Pollution Study, infants born to aeroallergen-sensitized and symptomatic parents were examined and underwent skin prick testing (SPT) annually to 15 aeroallergens from 1 to 4 years of age. Wheal circumferences were traced and scanned and areas were determined by computer planimetry. At 4 years, AER was defined as (1) at least 1 positive aeroallergen SPT result, (2) presence of sneezing and runny nose without a cold or influenza, and (3) nasal eosinophilia of at least 5%. Wheal areas at 1 to 3 years were analyzed for an association with AER compared with children without AR.
**Results:** At 4 years, 487 children completed rhinitis health histories, SPT, and nasal sampling. Ninety-nine children (22.8%) had AR. Thirty-eight children had AER (8.8% of total sample and 38.4% of AR sample, respectively). At 3 years, for every 1-mm$^2$ increase in *Penicillium* species (adjusted odds ratio 1.18, 95% confidence interval 1.06–1.32, $P = .002$) and maple (adjusted odds ratio 1.07, 95% confidence interval 1.01–1.13, $P = .02$), wheal area significantly increased the risk of AER at 4 years of age.
**Conclusion:** Allergic eosinophilic rhinitis was identified in 8.8% of children at 4 years of age. Age 3 years was the earliest that aeroallergen SPT wheal areas were predictive of AER. Skin testing at 3 years identifies children at risk for an AR phenotype with nasal eosinophilia.


## Introduction

Allergic inflammation is associated with tissue eosinophilia, which is a prominent finding in nasal mucosa of patients with allergic rhinitis (AR).[1] Nasal eosinophils correlate with nasal symptom severity in adults with seasonal AR.[2] In addition to reflecting inflammation within the upper airway, nasal eosinophilia is associated with sputum eosinophilia in patients with AR and concomitant asthma.[3] Nasal eosinophils can be objectively measured as a biomarker of allergic airway inflammation.[3] Nasal eosinophils correlated with chronic nasal symptoms in a cross-sectional study of Finnish children and adults, although their atopic status was unknown.[4] Currently, the percentage of young children with AR who have nasal eosinophilia is unknown.

It is unknown whether early skin prick testing (SPT) to aeroallergens can identify children with severe AR using an objective biomarker such as nasal eosinophils. Linking the magnitude of the wheal reaction younger in life to an objective biomarker, such as nasal eosinophilia, could be attractive in future intervention trials by identifying those children most susceptible to the later onset of

**Reprints:** Christopher D. Codispoti, MD, PhD, Division of Immunology, Microbiology and Allergy, Rush University Medical Center, 1725 W Harrison Ave, Suite 117, Chicago, IL 60612; E-mail: Christopher_D_Codispoti@rush.edu.
**Disclosure:** Dr Bernstein and Dr Lockey each received a co-investigating grant from the National Institute of Environmental Health Services. Dr Reponen has a cooperative agreement with the Centers for Disease Control and Prevention. Dr LeMasters received a grant from the National Institutes of Health (principal investigator). Dr Codispoti received grants from the National Institutes of Health.
**Funding:** This work was supported by National Institute of Environmental Health Services grants ES 11170 and ES 10957 and National Institute of Allergy and Infectious Disease grant AI 60515 T32.

http://dx.doi.org/10.1016/j.anai.2014.12.008
1081-1206/

severe AR symptoms. The hypothesis of this study was that specific aeroallergen wheal areas during the first 3 years of childhood would be associated with allergic eosinophilic rhinitis (AER) at 4 years of age. An association between wheal area by SPT at a young age and AER would reinforce a connection between early aeroallergen sensitization and childhood AR and provide important diagnostic information for earlier diagnosis of severe AR.

## Methods

### *Study Population*

The Cincinnati Childhood Allergy and Air Pollution Study (CCAAPS) strategy for recruiting infants at high risk for developing allergic disease has been published.[5,6] Birth records were obtained for infants born in greater Cincinnati and northern Kentucky. Parents were required to live nearer than 400 m or farther than 1,500 m from a major road to determine whether early traffic-related air pollution exposures were associated with allergic disease. However, the authors previously found that traffic-related air pollution is associated with wheezing but not with AR.[7,8] Of those parents living within the defined area, at least 1 parent reporting a symptom history of allergies or asthma was required for SPT eligibility. Symptomatic parents were invited to a screening visit and, after obtaining written informed consent that was approved by the University of Cincinnati institutional review board, underwent SPT to 15 aeroallergens. Aeroallergens in the screening SPT panel included eastern red cedar, American elm, maple mix, white oak, meadow fescue, timothy, short ragweed, house dust mite mix (*Dermatophagoides farinae* and *Dermatophagoides pteronyssinus*), German cockroach, cat, dog, and 4 mold allergens (*Alternaria alternata*, *Aspergillus fumigatus*, *Penicillium* species mix, and *Cladosporium* species; ALK-Abelló, Hørsholm, Denmark). These symptomatic parents who also were sensitive to at least 1 aeroallergen were invited to enroll their infant into the CCCAPS cohort.[5]

### *Clinical Visits*

At 1 year of age, parents brought their infants to CCAAPS clinics for clinical evaluation. The CCAAPS clinical staff interviewed the parents using questionnaires to obtain details on the infant's medical history and the home environmental history. Infants were examined and underwent SPT to the same 15 aeroallergens used in the parental screening panel in addition to cow's milk and hen's egg. The children returned to the CCAAPS clinics annually at 2, 3, and 4 years of age for repeat physical examination, SPT, and parental interview. At the year 4 visit, nasal epithelial smears also were obtained.

### *Quantitative Skin Prick Testing*

Skin prick testing was performed using a bifurcated needle coated with histamine dihydrochloride (10 mg/mL) as a positive control, 50% glycerinated human serum albumin–saline as a negative control, or 1 of the 17 test panel allergens.[9] Skin reactions were read 15 minutes after SPT. A positive reaction was noted if the diameter was at least 3 mm larger than the negative control in accordance with the most recent allergy diagnostic practice parameter published by the American Academy of Allergy, Asthma, and Immunology and American College of Allergy, Asthma, and Immunology.[9] All wheal and flare circumferences were traced with ink pen. The ink was absorbed by Transpore tape (3M, St Paul, Minnesota) and affixed to a labeled grid paper in the child's permanent record. These records were scanned and saved as true image files. The ink outlines of wheal circumferences were digitally retraced and the enclosed area was calculated using AutoCAD (Autodesk, Inc, San Rafael, California). For accuracy, these measurements were performed independently in duplicate by 2 independent individuals.

### *Nasal Cytology*

At 4 years of age, each inferior nasal turbinate was swabbed with a separate cotton applicator. The sample processing was adapted from a previously published protocol.[2,10,11] Cells were stained with Nasal Cytology Stain (Volu-Sol, Inc).[12] Only cells with an intact nucleus and cytoplasm were counted. The number of eosinophils was counted using 40× or 100× magnification until a maximum of 400 cells was counted. For quality control, a second scientist counted 10% of samples using a random block sampling procedure of each quartile. There was no significant difference between the cell counts of each scientist.

### *Health Outcomes*

At each annual visit, the parents were asked the International Study of Asthma and Allergies in Childhood (ISAAC) validated question, "In the past 12 months, has your child ever had a problem with sneezing, or a runny, or a blocked nose when he/she DID NOT have a cold or flu?"[13] AR was defined as a positive response to the ISAAC question and a positive SPT reaction to 1 of the 15 aeroallergens. AER, the primary outcome of this study, was defined as a positive response to the ISAAC question, a positive SPT reaction to 1 of the 15 aeroallergens, and more than 5% nasal eosinophils.[3] These AER cases were compared with children without nasal symptoms and negative SPT reactions to all 15 aeroallergens.

### *Exposure Assessments*

Before 1 year of age, the CCAAPS research staff visited the infant's home. The home's general characteristics, basement, and the infant's primary activity room and sleeping room were inspected for visible mold, water damage, and the general state of repair of each room. To determine the greatest component of endotoxin, (1-3)-$\beta$-D-glucan, and indoor allergen exposure, the infant's primary activity room, a 2-$m^2$ area of floor space, was vacuumed at a standard rate of 2 min/$m^2$.[14,15] The collected dust samples were filtered, desiccated, and stored at −20°C.[16] The dust samples were separated for measuring house dust endotoxin (endotoxin units per milligram of settled dust) and (1-3)-$\beta$-D-glucan (micrograms per gram of dust) by the limulus amebocyte lysate assay (Associates of Cape Cod, Inc, East Falmouth, Massachusetts).[17] Separate aliquots of settled dust were used for analysis of major cat allergen (Fel d 1), major dog allergen (Can f 1), major dust mite allergen (Der f 1), and major cockroach allergen (Bla g 1) by monoclonal sandwich enzyme-linked immunosorbent assay.[18–21]

### *Covariates*

Other covariates previously identified in the CCAAPS cohort as relevant for AR were evaluated for model inclusion and included ethnicity (non–African American vs African American), sex, annual household income (>$20,000 vs ≤$20,000), breastfeeding duration (months), number of children in the home (≥2 vs <2 children), season of birth, and the environmental covariates described earlier.[22] Hair cotinine levels were measured and used as an objective biomarker of tobacco smoke exposure at 2 years of age.[23]

### *Data Analysis*

The aeroallergen wheal areas at 1, 2, and 3 years of age were analyzed for associations to AER using logistic regression. The odds ratios (ORs) and 95% confidence intervals (CIs) reported were obtained from the profile likelihood ratio. Any allergen wheal area or covariate significantly associated with AER ($\alpha < 0.2$) was further evaluated in multivariate logistic regression. Home environmental

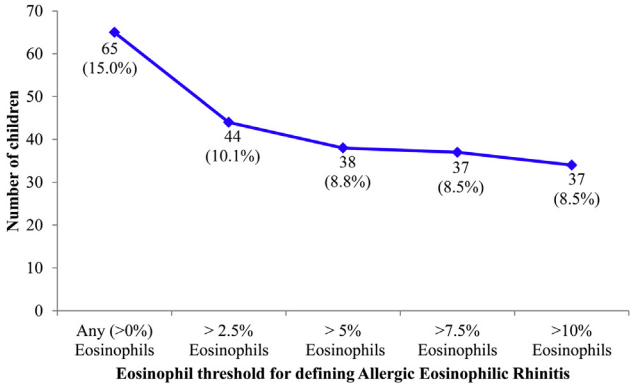


**Figure 1.** Sensitivity analysis with variation of the threshold for defining allergic eosinophilic rhinitis.

exposures (endotoxin, $\beta$-glucan, Fel d 1, Can f 1, Der p 1, and Bla g 1) were analyzed as continuous independent variables with thresholds defined by the turning points in the smooth plot that result from the use of a general additive model.[24] Independent exposures and covariates associated with AER at the 0.2 level were further investigated in a multivariate model. Variables in the multivariate model were eliminated by the "all subsets" method of selection, with the purpose of minimizing the log-likelihood ratio. A combined regression model that used informative predictors from regression models at 1, 2, and 3 years was developed. Analyses were performed using SAS 9.2 (SAS Institute, Cary, North Carolina).

## Results

### *Subjects*

The CCAAPS enrolled 762 infants and 636 (83.5%) were evaluated at 4 years of age. Of these, the parents of 478 children (75.2%) consented to nasal eosinophil sampling. Children whose parents did provide consent to nasal sampling were more likely to be non–African American and have higher paternal education, higher household income, no more than 2 children in the home, and lower elemental carbon attributable to traffic (ECAT) exposure (eTables 1 and 2). Of 478 children, 437 (91.4%) had technically interpretable nasal smears and these were more likely to be from households of higher income and lower ECAT exposure (eTables 3 and 4). Complete rhinitis response, SPT data, and interpretable nasal scraping data were available for 434 children.

### *Sensitivity Analyses*

To determine whether 5% was an acceptable cutoff for the definition of AER, the eosinophil threshold was varied over a range of possible limits. Figure 1 shows the number of children with AER as a function of the percentage of nasal eosinophils. Increasing the eosinophil threshold above 5% did not appreciably change the number of children with AER. In contrast, lowering the eosinophil threshold increased the number of children with AER at risk of losing phenotype specificity. Therefore, an eosinophil threshold of 5% was used in the study. This threshold is consistent with a previously published study and represents a more specific inflammatory phenotype of AR.[3] Of these 434 children, 119 (27.4%) had at least 5% nasal eosinophils. Of 119 children with at least 5% nasal eosinophils, 79 (66.4%) were sensitized to aeroallergens, and 49 (41.2%) had chronic nasal symptoms. Of the 49 children with at least 5% nasal eosinophils and chronic nasal symptoms, 38 (77.5%) had AER, representing 8.8% of the entire sample. The comparison group for AER includes children without AR.

**Table 1**
Unadjusted characteristics to allergic rhinitis with high eosinophils compared with combined phenotypes other than allergic rhinitis at 4 years old[a]

| Covariates | Frequency (%) | AER (n = 38/434, 8.8%) | All phenotypes other than AER (n = 335/434, 77.2%) | OR (95% CI) |
|---|---|---|---|---|
| Sex | | | | 2.81 (1.34–6.46)[c] |
| Girls | 193 (44.5) | 9 (4.7) | 156 (80.8) | |
| Boys | 241 (55.5) | 29 (12.0) | 179 (74.3) | |
| Race | | | | 0.90 (0.35–2.03) |
| NAA | 345 (79.5) | 31 (9.0) | 268 (77.7) | |
| AA | 89 (20.5) | 7 (7.9) | 67 (75.3) | |
| Maternal education | | | | 0.37 (0.17–0.76)[c] |
| College graduate | 213 (50.7) | 27 (12.7) | 161 (75.6) | |
| Some college or trade | 207 (49.3) | 10 (4.8) | 162 (78.3) | |
| Paternal education | | | | 0.71 (0.29–1.55) |
| Some college or trade | 298 (71.1) | 29 (9.7) | 232 (77.9) | |
| HS diploma or less | 121 (28.9) | 8 (6.6) | 90 (74.4) | |
| Household income | | | | 0.69 (0.20–1.83) |
| ≥$20,000 | 354 (84.7) | 33 (9.3) | 273 (77.1) | |
| <$20,000 | 64 (15.3) | 4 (6.3) | 48 (75.0) | |
| Season of birth | | | | |
| Winter | 151 (34.8) | 11 (7.3) | 116 (76.8) | — |
| Spring | 90 (20.7) | 6 (6.7) | 73 (81.1) | 0.87 (0.29–2.38) |
| Summer | 83 (19.1) | 8 (9.6) | 65 (78.3) | 1.30 (0.48–3.37) |
| Autumn | 110 (25.4) | 13 (11.8) | 81 (73.6) | 1.69 (0.72–4.04) |
| Breastfeeding duration (mo) | | | | |
| ≥4 | 202 (46.5) | 23 (11.4) | 153 (75.7) | — |
| <4 | 232 (53.5) | 15 (6.5) | 182 (78.5) | 0.55 (0.27–1.08)[b] |

Abbreviations: AA, African American; AER, allergic eosinophilic rhinitis; CI, confidence interval; HS, high school; NAA, non–African American; OR, odds ratio.
[a]In total, 434 children had complete questionnaire responses, skin prick testing data, and nasal cytology data at 4 years of age.
[b]$P < .2$.
[c]$P < .05$.
[d]$P < .003$.

### *Unadjusted Analyses*

The allergen wheal areas were analyzed for associations with AER after correcting for multiple comparisons. At 1 and 2 years of age, no allergen wheal area was significantly associated with AER. At 3 years, *Penicillium* species ($P = .04$), maple ($P = .02$), and elm ($P = .11$) wheal areas met the criteria for further analysis ($P < .2$) in the multivariable model with AER.

As presented in Table 1, the unadjusted OR of the children's characteristics that predicted AER and were included in the multivariable model were sex (OR 2.81, 95% CI 1.34–6.46, $P = .009$), maternal education level (OR 0.37, 95% CI 0.17–0.76, $P = .01$), and breastfeeding duration (OR 0.55, 95% CI 0.27–1.08, $P = .09$). The unadjusted ORs of environmental exposures meeting the inclusion criteria were only low, medium, and high house dust Fel d 1 levels (Table 2). ECAT showed a nonsignificant protective association. After further investigation, it was determined that a positive SPT reaction at 2 and 3 years acted as an intervening variable, thus necessitating its removal from further analysis.

### *Adjusted Analysis*

Table 3 lists the adjusted ORs (aORs) of allergen wheal area and covariates (children's characteristics and environmental exposures). At 3 years of age, *Penicillium* species (aOR 1.18, 95% CI 1.06–1.32, $P = .002$) and maple (aOR 1.07, 95% CI 1.01–1.13, $P = .02$) wheal areas were significantly associated with AER. In addition, elm showed a borderline association with AER (aOR 1.06, 95% CI 0.98–1.14, $P = .11$). Compared with the binary (positive or negative) measurement of aeroallergens, the *Penicillium* wheal area was more

**Table 2**
Unadjusted environmental exposure association to allergic rhinitis with high eosinophils compared with combined phenotypes other than allergic rhinitis at 4 years old[a]

| Environmental exposure | n (%) | AER (n = 38/434, 8.8%) | All phenotypes other than AER (n = 335/434, 77.2%) | OR (95% CI) |
|---|---|---|---|---|
| HDE (EU/mg dust) | | | | |
| <230 | 388 (89.4) | 32 (8.3) | 300 (77.3) | NC |
| 230–640 | 39 (9.0) | 6 (15.4) | 29 (74.4) | NC |
| ≥640 | 7 (1.6) | 0 (0) | 6 (85.7) | NC |
| β-Glucan (μg/g dust) | | | | |
| <60 | 272 (62.7) | 24 (8.8) | 205 (75.4) | 1.00 (0.98–1.02) |
| 60–170 | 116 (26.7) | 10 (8.6) | 95 (81.9) | 1.01 (0.97,1.03) |
| ≥33.12 | 46 (10.6) | 4 (8.7) | 35 (76.1) | 1.00 (0.98–1.02) |
| Fel d 1 (μg/mL) | | | | |
| <4.1 | 76 (17.5) | 3 (4.0) | 65 (85.5) | 3.38 (0.95–18.67)[b] |
| 4.1–148.4 | 230 (53.0) | 20 (8.7) | 179 (77.8) | 0.24 (0.04–1.09)[b] |
| ≥148.4 | 128 (29.5) | 15 (11.7) | 91 (71.1) | 1.64 (0.92 2.93)[b] |
| Der p 1 (μg/mL) | | | | |
| <54.6 | 337 (77.7) | 33 (9.8) | 257 (76.3) | 1.03 (0.79–1.34) |
| ≥54.6 | 97 (22.4) | 5 (5.2) | 78 (80.4) | 0.66 (0.28–1.37) |
| Can f 1 (μg/mL) | | | | |
| <0.74 | 210 (48.4) | 18 (8.6) | 161 (76.7) | 5.60 (0.03–>999) |
| 0.74–9.03 | 96 (22.1) | 12 (12.5) | 72 (75.0) | 0.19 (<0.01–19.78) |
| 9.03–221.4 | 95 (21.9) | 7 (7.4) | 75 (79.0) | 1.15 (0.33–4.06) |
| ≥221.4 | 33 (7.6) | 1 (3.0) | 27 (81.8) | 0.61 (0.28–1.31) |
| Bla g 1 (μg/mL) | | | | |
| <0.07 | 414 (95.4) | 37 (8.9) | 321 (77.5) | 0.58 (0.16–1.35) |
| ≥0.07 | 20 (4.6) | 1 (5.0) | 14 (70.0) | 1.65 (0.87–5.34) |
| Year 2 cotinine (ng/mg hair) | | | | |
| <0.11 | 301 (69.4) | 26 (8.6) | 235 (78.1) | 1.02 (0.45–2.31) |
| 0.11–0.67 | 116 (26.7) | 12 (10.3) | 86 (74.1) | 0.98 (0.61–1.56) |
| ≥0.67 | 17 (3.9) | 0 (0) | 14 (82.4) | NC |
| Year 1 ECAT exposure (μg/m$^3$) | | | | |
| ≤0.32 | 298 (68.7) | 31 (10.4) | 226 (75.8) | — |
| >0.32 | 136 (31.3) | 7 (5.2) | 109 (80.2) | 0.47 (0.19–1.04)[b] |
| Children in home at 12 mo | | | | |
| ≥2 | 141 (32.5) | 9 (6.4) | 113 (80.1) | |
| <2 | 293 (67.5) | 29 (9.9) | 222 (75.8) | 1.64 (0.78–3.78) |
| Stays in daycare-like facility for ≥8 h during first year | | | | |
| No | 277 (64.9) | 28 (10.1) | 214 (77.3) | |
| Yes | 150 (35.1) | 8 (5.3) | 116 (77.3) | 0.53 (0.22–1.14) |
| Colds at 12 mo | | | | |
| <7 | 396 (91.2) | 35 (8.8) | 307 (77.5) | |
| ≥7 | 38 (8.8) | 3 (7.9) | 28 (73.7) | 0.94 (0.22–2.83) |

Abbreviations: AER, allergic eosinophilic rhinitis; CI, confidence interval; ECAT, elemental carbon attributable to traffic; EU, endotoxin units; HDE, house dust endotoxin; HS, high school; NC, not calculable; OR, odds ratio.
[a]In total, 434 children had complete questionnaire responses, skin prick testing data; and nasal cytology date at 4 years old. Not listed are children with allergic rhinitis without high nasal eosinophil counts (n = 61).
[b]$P < .2$.

precise (smaller CIs) and more significantly associated with AER. The 2 tree measurements also were more precise (smaller CIs) compared with the binary values, but the significance levels were comparable.

These 3 informative allergen wheal areas at 3 years of age were summed and showed a significant linear relation to AER (OR 1.1, 95% CI 1.02–1.09, $P = .003$). These summed wheal areas were compared with those in children who exhibited negative reactions for all 3 allergens. As shown in Figure 2, each percentile showed a dose-dependent increased risk of AER. Children with a sum of all 3 allergen wheal areas in the 25th percentile (OR 3.6, 95% CI 1.12–10.47, $P = .03$), >25th to <75th percentile (OR 4.1, 95% CI 1.78–9.01, $P = .0001$), and 75th percentile (OR 10.7, 95% CI 3.43–34.20, $P < .0001$) had an increased risk of AER. Male sex (OR 2.5, 95% CI 1.07–6.44, $P = .04$) and higher maternal education (OR 0.38, 95% CI 0.16–0.87, $P = .03$) also were significantly associated with AER.

**Table 3**
Adjusted odds ratio and 95% confidence intervals of allergen wheal area at each year associated with allergic eosinophilic rhinitis at 4 years old[a]

| Allergens | At 3 y old | | | |
|---|---|---|---|---|
| | Allergen wheal area | | Allergen binary value | |
| | aOR (95% CI) | *P* value | aOR (95% CI) | *P* value |
| Elm | 1.06 (0.98–1.14) | .11 | 2.93 (0.68–10.92) | .12 |
| Maple | 1.07 (1.01–1.13) | .02 | 3.72 (1.11–11.53) | .03 |
| *Penicillium* species | 1.18 (1.06–1.32) | .002 | 4.29 (0.99–15.86) | .03 |

Abbreviations: aOR, adjusted odds ratio; CI, confidence interval.
[a]Sex and maternal education were included in the model for 3 years of age to improve model fit.

## Discussion

Previously, little has been reported about the etiology and significance of nasal eosinophilia in children. In a small study of 20 adolescent Italian children with perennial AR, nasal eosinophils were significantly associated with a nasal total symptom score showing a significant inverse association with nasal airflow.[25] A larger study of 160 Chinese preschool children with perennial AR found a significant association of nasal eosinophil grade with total nasal symptom score.[26] The present study is the first to

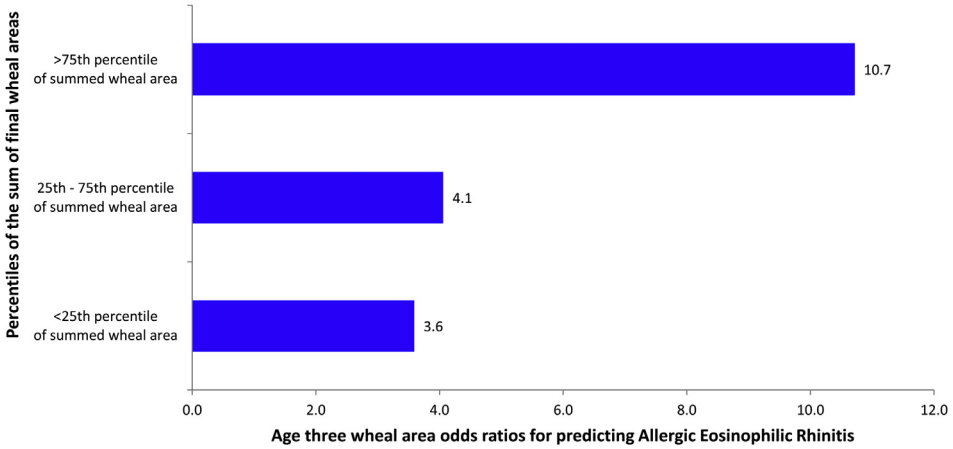


**Figure 2.** Odds ratios of developing allergic eosinophilic rhinitis at 4 years of age by percentiles of total important allergen wheal 3 areas from multivariate regression model at 3 years of age. Allergen wheal areas that were included in the final multivariate model at 3 years were *Penicillium* species, maple, and elm.

demonstrate that early aeroallergen sensitization is associated with AR in children who also have a large number of nasal eosinophils (AER) at 4 years of age in a large North American cohort. Of the 434 children in this study, 38 (8.8%) had AER.

The present study was an investigation of early aeroallergen sensitization measured by allergen wheal area in predicting AER in high-risk children. These findings are the first to demonstrate that at as young as 3 years aeroallergen wheal areas are significantly associated with AER at 4 years. These results indicate that especially *Penicillium* and maple wheal areas (with elm wheal showing borderline significance) at 3 years are most predictive of AER. Those children with the largest sum of these 3 informative wheal areas had an approximate 11-fold increase in risk of AER. However, at 1 and 2 years, after controlling for relevant covariates, no significant associations were observed with allergen wheal areas and AER at 4 years, indicating that in this cohort earlier aeroallergen sensitization is less informative for AER at 4 years. The authors previously reported that *Penicillium* species is the most prevalent measureable fungal species in indoor and outdoor environments.[27] Recent evidence has suggested that *Penicillium* species is important to allergic respiratory disorders. In a murine model of asthma, *Penicillium* extract induced more vigorous inflammatory response in bronchoalveolar lavage fluid compared with house dust mite extract.[28] In Puerto Rican inner-city children, *Penicillium* mold count in a child's bedroom was associated with increased frequency of asthma symptoms, although no tests of sensitization were performed.[29] Another study found that *Penicillium* sensitization increases with age in children with asthma.[30] More recent studies have demonstrated that the presence of *Penicillium* species in the air is associated with infant wheezing.[31] In school-age children with asthma, *Penicillium* sensitization and exposure increased the risk of wheezing and asthma severity score.[32] *Penicillium* sensitization frequency in the present cohort also increased at 1 year (n = 8), 2 years (n = 12), and 3 years (n = 25) of age, respectively. Less is known on how maple allergen exposure and sensitization contributes to allergic respiratory disease. Maple is a predominant tree species and a major aeroallergen (a sensitizer in >50% of patients with springtime seasonal AR) in the greater Cincinnati and northern Kentucky area.[33] Maple pollen levels have been correlated with asthma hospital admissions in Canada, and levels have been positively correlated with asthma hospital admissions in Portugal.[34,35] In previous work, the authors found that tree sensitivity at 1 year of age was associated with AR at 3 years.[22] This association of *Penicillium* and maple sensitization with AER, a possible severe AR phenotype, warrants further investigation.

The strengths of this prospective study of early childhood allergic disease are its repeated annual health interviews, physical examinations, allergen SPT, and use of eosinophils to measure AR severity. The nasal eosinophils provide an objective biomarker of a more specific phenotype of AER. This phenotype could be useful as a severe phenotype for future intervention trials, especially for high-risk children (ie, those born to parents with atopy). The large sample of 4-year-old children allowed for an investigation of less prevalent outcomes, such as AER. The use of wheal areas as a continuous measurement increased the power to detect an association with AER.

As with all studies, there were limitations. The children underwent SPT to relevant aeroallergens for the greater Cincinnati metropolitan area, which could limit generalizability to other regions. The importance of different sets of aeroallergen SPT results in other regions will need to be determined. Children whose parents consented to nasal scraping were more often non–African American. However, when analyzing whether participating children providing interpretable nasal eosinophils differed from the larger cohort, there was no significant difference in race. Also, this study did not measure eosinophils at younger ages. Therefore, no assumptions can be made regarding whether earlier testing might be predictive of earlier AER.

In summary, this study found that only a few age-specific allergen wheal areas were associated with AER at 4 years of age. None were predictive before 3 years, but *Penicillium* and maple wheal areas at 3 years were significantly associated with AER at 4 years. Hence, 3 years of age could be an important time to begin SPT in children, especially those from high-risk families. Children with the largest sum of wheal areas for *Penicillium* species, maple, and elm at 3 years old were at greatest risk of AER at 4 years. Linking the severity of early childhood allergen sensitization to a severe AR phenotype is useful for defining future clinical and study groups for longitudinal observation and intervention studies.

## Acknowledgements

The authors thank Stacy Langemeyer, BS, who contributed in the collection, quantifying, and storing the nasal eosinophils.

## Supplementary Data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.anai.2014.12.008.

## References


[1] Stone KD, Prussin C, Metcalfe DD. IgE, mast cells, basophils, and eosinophils. *J Allergy Clin Immunol*. 2010;125(suppl 2):S73–S80.

[2] Ciprandi G, Vizzaccaro A, Cirillo I, Tosca M, Massolo A, Passalacqua G. Nasal eosinophils display the best correlation with symptoms, pulmonary function and inflammation in allergic rhinitis. *Int Arch Allergy Immunol*. 2005;136:266–272.

[3] Amorim MM, Araruna A, Caetano LB, Cruz AC, Santoro LL, Fernandes AL. Nasal eosinophilia: an indicator of eosinophilic inflammation in asthma. *Clin Exp Allergy*. 2010;40:867–874.

[4] Malmberg H. Symptoms of chronic and allergic rhinitis and occurrence of nasal secretion granulocytes in university students, school children and infants. *Allergy*. 1979;34:389–394.

[5] LeMasters GK, Wilson K, Levin L, et al. High prevalence of aeroallergen sensitization among infants of atopic parents. *J Pediatr*. 2006;149:505–511.

[6] Ryan PH, LeMasters G, Biagini J, et al. Is it traffic type, volume, or distance? Wheezing in infants living near truck and bus traffic. *J Allergy Clin Immunol*. 2005;116:279–284.

[7] Ryan PH, Bernstein DI, Lockey J, et al. Exposure to traffic-related particles and endotoxin during infancy is associated with wheezing at age 3 years. *Am J Respir Crit Care Med*. 2009;180:1068–1075.

[8] Codispoti CD, LeMasters GK, Levin L, et al. Aeroallergen wheal area especially for molds at ages two and three predicts allergic rhinitis at age four: the Cincinnati Childhood Allergy and Air Pollution Study (CCAAPS). *J Allergy Clin Immunol*. 2013;131(suppl):AB237.

[9] Bernstein IL, Li JT, Bernstein DI, et al. Allergy diagnostic testing: an updated practice parameter. *Ann Allergy Asthma Immunol*. 2008;100(suppl 3):S1–S148.

[10] Ciprandi G, Cirillo I, Vizzaccaro A, Milanese M, Tosca MA. Nasal obstruction in patients with seasonal allergic rhinitis: relationships between allergic inflammation and nasal airflow. *Int Arch Allergy Immunol*. 2004;134:34–40.

[11] Ciprandi G, Tosca M, Ricca V, et al. Cetirizine treatment of allergic cough in children with pollen allergy. *Allergy*. 1997;52:752–754.

[12] Kagan R, Hayami D, Joseph L, St Pierre Y, Clarke AE. The predictive value of a positive prick skin test to peanut in atopic, peanut-naive children. *Ann Allergy Asthma Immunol*. 2003;90:640–645.

[13] Asher MI, Keil U, Anderson HR, et al. International Study of Asthma and Allergies in Childhood (ISAAC): rationale and methods. *Eur Respir J*. 1995;8:483–491.

[14] Iossifova YY, Reponen T, Bernstein DI, et al. House dust (1-3)-beta-D-glucan and wheezing in infants. *Allergy*. 2007;62:504–513.

[15] Hamilton RG. Assessment of indoor allergen exposure. *Curr Allergy Asthma Rep*. 2005;5:394–401.

[16] Reponen T, Vesper S, Levin L, et al. High environmental relative moldiness index during infancy as a predictor of asthma at 7 years of age. *Ann Allergy Asthma Immunol*. 2011;107:120–126.

[17] Milton DK, Johnson DK, Park JH. Environmental endotoxin measurement: interference and sources of variation in the Limulus assay of house dust. *Am Ind Hyg Assoc J*. 1997;58:861–867.

[18] Chapman MD, Aalberse RC, Brown MJ, Platts-Mills TA. Monoclonal antibodies to the major feline allergen Fel d I. II. Single step affinity purification of Fel d I, N-terminal sequence analysis, and development of a sensitive two-site immunoassay to assess Fel d I exposure. *J Immunol*. 1988;140:812–818.

[19] Chapman MD, Heymann PW, Wilkins SR, Brown MJ, Platts-Mills TA. Monoclonal immunoassays for major dust mite (Dermatophagoides) allergens, Der p I and Der f I, and quantitative analysis of the allergen content of mite and house dust extracts. *J Allergy Clin Immunol*. 1987;80:184–194.

[20] Pollart SM, Smith TF, Morris EC, Gelber LE, Platts-Mills TA, Chapman MD. Environmental exposure to cockroach allergens: analysis with monoclonal antibody-based enzyme immunoassays. *J Allergy Clin Immunol*. 1991;87:505–510.

[21] Schou C, Hansen GN, Lintner T, Lowenstein H. Assay for the major dog allergen, Can f I: investigation of house dust samples and commercial dog extracts. *J Allergy Clin Immunol*. 1991;88:847–853.

[22] Codispoti CD, Levin L, LeMasters GK, et al. Breast-feeding, aeroallergen sensitization, and environmental exposures during infancy are determinants of childhood allergic rhinitis. *J Allergy Clin Immunol*. 2010;125:1054–1060 e1051.

[23] Biagini Myers JM, Hershey GK, Deka R, et al. Asking the right questions to ascertain early childhood secondhand smoke exposures. *J Pediatr*. 2012;160:1050–1051.

[24] Harrell J, Frank E. *Regression Modeling Strategies: With Application to Linear Models, Logistic Regression, and Survival Analysis*. New York: Springer-Verlag; 2001.

[25] Ciprandi G, Marseglia GL, Klersy C, Tosca MA. Relationships between allergic inflammation and nasal airflow in children with persistent allergic rhinitis due to mite sensitization. *Allergy*. 2005;60:957–960.

[26] Chen ST, Sun HL, Lu KH, Lue KH, Chou MC. Correlation of immunoglobulin E, eosinophil cationic protein, and eosinophil count with the severity of childhood perennial allergic rhinitis. *J Microbiol Immunol Infect*. 2006;39:212–218.

[27] Osborne M, Reponen T, Adhikari A, et al. Specific fungal exposures, allergic sensitization, and rhinitis in infants. *Pediatr Allergy Immunol*. 2006;17:450–457.

[28] Ward MD, Chung YJ, Copeland LB, Doerfler DL. A comparison of the allergic responses induced by Penicillium chrysogenum and house dust mite extracts in a mouse model. *Indoor Air*. 2010;20:380–391.

[29] Turyk M, Curtis L, Scheff P, et al. Environmental allergens and asthma morbidity in low-income children. *J Asthma*. 2006;43:453–457.

[30] Chou H, Chang CY, Tsai JJ, et al. The prevalence of IgE antibody reactivity against the alkaline serine protease major allergen of Penicillium chrysogenum increases with the age of asthmatic patients. *Ann Allergy Asthma Immunol*. 2003;90:248–253.

[31] Behbod B, Sordillo JE, Hoffman EB, et al. Wheeze in infancy: protection associated with yeasts in house dust contrasts with increased risk associated with yeasts in indoor air and other fungal taxa. *Allergy*. 2013;68:1410–1418.

[32] Gent JF, Kezik JM, Hill ME, Tsai E, Li DW, Leaderer BP. Household mold and dust allergens: exposure, sensitization and childhood asthma morbidity. *Environ Res*. 2012;118:86–93.

[33] White JF, Levin L, Villareal M, et al. Lack of correlation between regional pollen counts and percutaneous reactivity to tree pollen extracts in patients with seasonal allergic rhinitis. *Ann Allergy Asthma Immunol*. 2005;94:240–246.

[34] Dales RE, Cakmak S, Judek S, Coates F. Tree pollen and hospitalization for asthma in urban Canada. *Int Arch Allergy Immunol*. 2008;146:241–247.

[35] Ribeiro H, Oliveira M, Ribeiro N, et al. Pollen allergenic potential nature of some trees species: a multidisciplinary approach using aerobiological, immunochemical and hospital admissions data. *Environ Res*. 2009;109:328–333.

**eTable 1**
Characteristic differences between children whose parents consented to nasal epithelium scraping and those whose parents did not

| Covariates | n (%) | Children NOT consented to nasal sampling, n (%) | Children consented to nasal sampling, n (%) | OR (95% CI) |
|---|---|---|---|---|
| Total | 634 (100.0) | 156 (24.6) | 478 (75.4) | |
| Allergic rhinitis | | | | 1.12 (0.72–1.76) |
| All other phenotypes | 495 (78.1) | 124 (25.1) | 371 (75.0) | |
| Yes | 139 (21.9) | 32 (23.0) | 107 (77.0) | |
| Sex | | | | 0.99 (0.69–1.42) |
| Girls | 290 (45.7) | 97 (33.5) | 193 (66.6) | |
| Boys | 344 (54.3) | 103 (29.9) | 241 (70.1) | |
| Race | | | | 0.63 (0.42–0.95)[b] |
| NAA | 492 (77.6) | 147 (29.9) | 345 (70.1) | |
| AA | 142 (22.4) | 53 (37.3) | 89 (62.7) | |
| Maternal education | | | | 0.89 (0.61–1.28) |
| College graduate | 307 (50.0) | 94 (30.6) | 213 (69.4) | |
| Some college/trade | 307 (50.0) | 100 (32.6) | 207 (67.4) | |
| Paternal education | | | | 0.75 (0.51–1.12)[a] |
| Some college/trade | 425 (69.3) | 127 (29.9) | 298 (70.1) | |
| HS diploma or less | 188 (30.7) | 67 (35.6) | 121 (64.4) | |
| Household income | | | | 0.54 (0.35–0.85)[b] |
| ≥$20,000 | 502 (82.3) | 148 (29.5) | 354 (70.5) | |
| <$20,000 | 108 (17.7) | 44 (40.7) | 64 (59.3) | |
| Season of birth | | | | 0.95 (0.65–1.42) |
| Winter | 209 (33.0) | 58 (27.8) | 151 (72.3) | |
| Spring | 140 (22.1) | 50 (35.7) | 90 (64.3) | |
| Summer | 138 (21.8) | 55 (39.9) | 83 (60.1) | |
| Autumn | 147 (23.2) | 37 (25.2) | 110 (74.8) | |
| Breastfeeding duration (mo) | | | | 0.90 (0.62–1.29) |
| ≥4 | 289 (45.6) | 87 (30.1) | 202 (69.9) | |
| <4 | 345 (54.4) | 113 (32.8) | 232 (67.3) | |
| Children in home at 12 mo | | | | 0.87 (0.58–1.29) |
| ≥3 | 197 (31.1) | 45 (22.8) | 152 (77.2) | |
| <3 | 437 (68.9) | 111 (25.4) | 326 (74.6) | |
| Stays in daycare-like facility for ≥8 h during first year | | | | 0.83 (0.57–1.22) |
| No | 399 (64.2) | 92 (23.1) | 307 (76.9) | |
| Yes | 223 (35.9) | 59 (26.5) | 164 (73.5) | |
| Colds at 12 mo | | | | 0.90 (0.49–1.76) |
| <7 | 581 (91.6) | 142 (24.4) | 439 (75.6) | |
| ≥7 | 53 (8.4) | 14 (26.4) | 39 (73.6) | |

Abbreviations: AA, African American CI, confidence interval; HS, high school; NAA, non–African American; OR, odds ratio.
[a] $P < .2$.
[b] $P < .05$.

**eTable 2**
Environmental exposure differences between children whose parents consented to nasal epithelium scraping and those whose parents did not

| Environmental exposure | n (%) | Children NOT consented to nasal sampling, n (%) | Children consented to nasal sampling, n (%) | OR (95% CI) |
|---|---|---|---|---|
| Total | 634 (100.0) | 156 (24.6) | 478 (75.4) | |
| Allergic rhinitis | | | | 1.12 (0.72–1.76) |
| All other phenotypes | 495 (78.1) | 124 (25.1) | 371 (75.0) | |
| Yes | 139 (21.9) | 32 (23.0) | 107 (77.0) | |
| HDE (EU/mg dust) | | | | |
| <230 | 567 (89.4) | 179 (31.6) | 388 (68.4) | 1.00 (1.00–1.00) |
| 230–640 | 57 (9.0) | 18 (31.6) | 39 (68.4) | 1.00 (0.99–1.01) |
| ≥640 | 10 (1.6) | 3 (30.0) | 7 (70.0) | 1.00 (0.97–1.02) |
| β-Glucan (μg/g dust) | | | | |
| <60 | 401 (63.3) | 129 (32.2) | 272 (67.8) | 1.00 (0.99–1.01) |
| 60–170 | 159 (25.1) | 43 (27.0) | 116 (73.0) | 1.00 (0.98–1.01) |
| ≥33.12 | 74 (11.7) | 28 (37.8) | 46 (62.2) | 1.00 (0.99–1.01) |
| Fel d 1 (μg/mL) | | | | |
| <4.1 | 113 (17.8) | 37 (32.7) | 76 (67.3) | 1.47 (0.85–2.53)[a] |
| 4.1–148.4 | 337 (53.2) | 107 (31.8) | 230 (68.3) | 0.64 (0.32–1.27) |
| ≥148.4 | 184 (29.0) | 56 (30.4) | 128 (69.6) | 1.07 (0.76–1.49) |
| Der p 1 (μg/mL) | | | | |
| <54.6 | 485 (76.5) | 148 (30.5) | 337 (69.5) | 1.16 (1.00–1.35) |
| ≥54.6 | 149 (23.5) | 52 (34.9) | 97 (65.1) | 0.66 (0.47–0.95) |
| Can f 1 (μg/mL) | | | | |
| <0.74 | 295 (46.5) | 85 (28.8) | 210 (71.2) | 4.04 (0.22–63.46) |
| 0.74–9.03 | 147 (23.2) | 51 (34.7) | 96 (65.3) | 0.27 (0.02–3.59) |
| 9.03–221.4 | 149 (23.5) | 54 (36.2) | 95 (63.8) | 0.87 (0.46–1.62) |
| ≥221.4 | 43 (6.8) | 10 (23.3) | 33 (76.7) | 1.11 (0.74–1.69) |
| Bla g 1 (μg/mL) | | | | |
| <0.07 | 612 (96.5) | 198 (32.4) | 414 (67.7) | 0.68 (0.47–0.99)[b] |
| ≥0.07 | 22 (3.5) | 2 (9.1) | 20 (90.9) | 1.30 (1.03–1.63)[b] |
| Year 2 cotinine (ng/mg hair) | | | | |
| <0.11 | 447 (70.5) | 115 (25.7) | 332 (74.3) | 0.63 (0.40,0.98)[b] |
| 0.11–0.67 | 160 (25.2) | 33 (20.6) | 127 (79.4) | 1.43 (1.12,1.85)[b] |
| ≥0.67 | 27 (4.3) | 8 (29.6) | 19 (70.4) | — |
| Year 1 ECAT exposure (μg/m$^3$) | | | | 0.76 (0.54–1.08)[a] |
| ≤0.32 | 423 (66.7) | 95 (22.5) | 328 (77.5) | |
| >0.32 | 211 (33.3) | 61 (28.9) | 150 (71.1) | |

Abbreviations: AER, allergic eosinophilic rhinitis; CI, confidence interval; ECAT, elemental carbon attributable to traffic; EU, endotoxin units; HDE, house dust endotoxin; HS, high school; OR, odds ratio.
[a] $P < .2$.
[b] $P < .05$.

**eTable 3**
Demographic characteristic differences between children with interpretable (non-censored) nasal samples and children without interpretable nasal smear information

| Covariates | Frequency (%) | Children without interpretable nasal smears, n (%) | Children with interpretable nasal smears, n (%) | OR (95% CI) |
|---|---|---|---|---|
| Total | 634 (100.0) | 200 (31.6) | 434 (68.5) | |
| Allergic rhinitis | | | | 1.18 (0.79–1.80) |
| All other phenotypes | 495 (78.1) | 160 (32.3) | 335 (67.7) | |
| Yes | 139 (21.9) | 40 (28.8) | 99 (71.2) | |
| Sex | | | | 1.18 (0.84–1.65) |
| Female | 290 (45.7) | 97 (33.5) | 193 (66.6) | |
| Male | 344 (54.3) | 103 (29.9) | 241 (70.1) | |
| Race | | | | 0.72 (0.49–1.06) |
| NAA | 492 (77.6) | 147 (29.9) | 345 (70.1) | |
| AA | 142 (22.4) | 53 (37.3) | 89 (62.3) | |
| Maternal education | | | | 0.91 (0.65–1.28) |
| College graduate | 307 (50.0) | 94 (30.6) | 213 (69.4) | |
| Some college or trade | 307 (50.0) | 100 (32.8) | 207 (67.4) | |
| Paternal education | | | | 0.77 (0.54–1.11) |
| Some college or trade | 425 (69.3) | 127 (29.9) | 298 (70.1) | |
| HS diploma or less | 188 (30.7) | 67 (35.6) | 121 (64.4) | |
| Household income | | | | 0.61 (0.40–0.94)[a] |
| ≥$20,000 | 502 (82.3) | 148 (29.5) | 354 (70.5) | |
| <$20,000 | 108 (17.7) | 44 (40.7) | 64 (59.3) | |
| Season of birth | | | | |
| Winter | 209 (33.0) | 58 (27.8) | 151 (72.3) | |
| Spring | 140 (22.1) | 50 (35.7) | 90 (64.3) | 0.69 (0.44–1.10) |
| Summer | 138 (21.8) | 55 (39.9) | 83 (60.1) | 0.58 (0.37–0.91)[a] |
| Autumn | 147 (23.2) | 37 (25.2) | 110 (74.8) | 1.14 (0.71–1.85) |
| Breastfeeding duration (mo) | | | | 0.88 (0.63–1.24) |
| ≥4 | 289 (45.6) | 87 (30.1) | 202 (69.9) | |
| <4 | 345 (54.4) | 113 (32.8) | 232 (67.3) | |
| Children in home at 12 mo | | | | 0.81 (0.56–1.16) |
| ≥3 | 197 (31.1) | 56 (28.4) | 141 (71.6) | |
| <3 | 437 (68.9) | 144 (33.0) | 293 (67.1) | |
| Stays in daycare-like facility for ≥8 h during first year | | | | 0.91 (0.64–1.29) |
| No | 399 (64.2) | 122 (30.6) | 277 (69.4) | |
| Yes | 223 (35.9) | 73 (32.7) | 150 (67.3) | |
| Colds during year 1 | | | | 1.18 (0.65–2.27) |
| <7 | 581 (91.6) | 185 (31.8) | 396 (68.2) | |
| ≥7 | 53 (8.4) | 15 (28.3) | 38 (71.7) | |

Abbreviations: AA, African American CI, confidence interval; HS, high school; NAA, non–African American; OR, odds ratio.
[a] $P < .05$.

**eTable 4**
Environmental exposure differences between children with interpretable (non-censored) nasal samples and children without interpretable nasal smear information

| Covariates | Frequency (%) | Children without interpretable nasal smear, n (%) | Children with interpretable nasal smear, n (%) | OR (95% CI) |
|---|---|---|---|---|
| Total | 634 (100.0) | 200 (31.6) | 434 (68.5) | |
| Allergic rhinitis | | | | 1.18 (0.79–1.80) |
| All other phenotypes | 495 (78.1) | 160 (32.3) | 335 (67.7) | |
| Yes | 139 (21.9) | 40 (28.8) | 99 (71.2) | |
| HDE (EU/mg dust) | | | | |
| <230 | 567 (89.4) | 179 (31.6) | 388 (68.4) | 1.00 (1.00–1.00) |
| 230–640 | 57 (9.0) | 18 (31.6) | 39 (68.4) | 1.00 (1.00–1.01) |
| ≥640 | 10 (1.6) | 3 (30.0) | 7 (70.0) | 1.00 (0.97–1.01) |
| $\beta$-Glucan ($\mu$g/g dust) | | | | |
| <60 | 401 (63.3) | 129 (32.2) | 272 (67.8) | 1.00 (0.99–1.01) |
| 60–170 | 159 (25.1) | 43 (27.0) | 116 (73.0) | 1.00 (0.99–1.01) |
| ≥33.12 | 74 (11.7) | 28 (37.8) | 46 (62.2) | 1.00 (0.99–1.01) |
| Fel d 1 ($\mu$g/mL) | | | | |
| <4.1 | 113 (17.8) | 37 (32.7) | 76 (67.3) | 1.24 (0.74–2.05) |
| 4.1–148.4 | 337 (53.2) | 107 (31.8) | 230 (68.3) | 0.83 (0.44–1.57) |
| ≥148.4 | 184 (29.0) | 56 (30.4) | 128 (69.6) | 0.89 (0.65–1.22) |
| Der p 1 ($\mu$g/mL) | | | | |
| <54.6 | 485 (76.5) | 148 (30.5) | 337 (69.5) | 1.17 (1.02,1.34)[b] |
| ≥54.6 | 149 (23.5) | 52 (34.9) | 97 (65.1) | 0.64 (0.46,0.89)[b] |
| Can f 1 ($\mu$g/mL) | | | | |
| <0.74 | 295 (46.5) | 85 (28.8) | 210 (71.2) | 2.78 (0.18,38.49) |
| 0.74–9.03 | 147 (23.2) | 51 (34.7) | 96 (65.3) | 0.38 (0.04–4.28) |
| 9.03–221.4 | 149 (23.5) | 54 (36.2) | 95 (63.8) | 0.87 (0.48–1.56) |
| ≥221.4 | 43 (6.8) | 10 (23.3) | 33 (76.7) | 1.21 (0.83–1.78) |
| Bla g 1 ($\mu$g/mL) | | | | |
| <0.07 | 612 (96.5) | 198 (32.4) | 414 (67.7) | 0.80 (0.54–1.17) |
| ≥0.07 | 22 (3.5) | 2 (9.1) | 20 (90.9) | 1.18 (0.92–1.49) |
| Year 2 cotinine (ng/mg hair) | | | | |
| <0.11 | 447 (70.5) | 146 (32.7) | 301 (67.3) | 0.81 (0.54–1.21) |
| 0.11–0.67 | 160 (25.2) | 44 (27.5) | 116 (72.5) | 1.21 (0.96–1.52) |
| ≥0.67 | 27 (4.3) | 10 (37.0) | 17 (63.0) | — |
| Year 1 ECAT exposure ($\mu$g/m$^3$) | | | | 0.76 (0.54–1.08)[a] |
| ≤0.32 | 423 (66.7) | 125 (29.6) | 298 (70.5) | |
| >0.32 | 211 (33.3) | 75 (35.6) | 136 (64.5) | |

Abbreviations: AER, allergic eosinophilic rhinitis; CI, confidence interval; ECAT, elemental carbon attributable to traffic; EU, endotoxin units; HDE, house dust endotoxin; HS, high school; OR, odds ratio.
[a] $P < .2$.
[b] $P < .05$.