Published in final edited form as:

Ann Allergy Asthma Immunol. 2015 March ; 114(3): 193–198.e4. doi:10.1016/j.anai.2014.12.008.

Early-life mold and tree sensitivity is associated with allergic eosinophilic rhinitis at age four

Christopher D. Codispoti, MD, PhD^{a,b,d}, David I. Bernstein, MD^a, Linda Levin, PhD^b, Tiina Reponen, PhD^b, Patrick H. Ryan, PhD^{b,c}, Jocelyn M Biagini Myers, PhD^d, Manuel Villareal, MD^a, Jeff Burkle, BS^b, Zana Lummus, PhD^a, James E. Lockey, MD, MS^{a,b}, Gurjit K. Khurana Hershey, MD, PhD^c, and Grace K. LeMasters, PhD^b

^athe Department of Internal Medicine, Divisions of Immunology/Allergy and Pulmonary Medicine, University of Cincinnati

^bthe Department of Environmental Health, University of Cincinnati

^cthe Division of Asthma Research, Children's Hospital Medical Center

^dthe Division of Immunology and Microbiology, Rush University Medical Center

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 AR patients with concomitant asthma.³ Nasal eosinophils can be objectively measured as a biomarker allergic airway inflammation.³ Nasal eosinophils correlated with chronic nasal symptoms in a cross-sectional study of Finnish children and adults, however their atopic status was unknown.⁴ Currently, it is unknown the percentage of young children with AR that have nasal eosinophilia.

It is unknown if early skin prick testing to aeroallergens can identify children with severe AR using an objective biomarker such as nasal eosinophils. Linking the magnitude of the wheal reaction earlier in life to an objective biomarker, such as nasal eosinophilia, may be attractive in future intervention trials by identifying those children most susceptible to the later onset of severe AR symptoms. The hypothesis of this study is that specific aeroallergen wheal area(s) in the first three years of childhood is associated with allergic eosinophilic rhinitis (AER) at age four. An association between early SPT wheal area and AER

© 2014 American College of Allergy, Asthma Immunology. Elsevier Inc. All rights reserved.

Correspondence address: Christopher D. Codispoti, MD, PhD, Division of Immunology, Microbiology and Allergy, Rush University Medical Center, 1725 W. Harrison Ave, Suite 117, Chicago, IL 60612, Telephone: (312) 942-6296, Fax: (312) 563-2201, Christopher_d_Codispoti@rush.edu, Cincinnati, Ohio, and Chicago, Illinois

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

reinforces a connection between early aeroallergen sensitization and childhood AR and also provides 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 have been previously published.^{5,6} Birth records were obtained for infants born in Greater Cincinnati and Northern Kentucky. Parents were required to live either less than 400 meters or more than 1500 meters from a major road in order to determine if early traffic-related air pollution exposures is associated with allergic disease. However, we have previously found that though traffic-related air pollution is associated with wheezing but not AR.^{7,8} Of those parents living within the defined area, at least one parent reporting a symptom history of allergies or asthma was required for skin testing eligibility. Symptomatic parents were invited to a screening visit and after obtaining written informed consent that was approved by University of Cincinnati Institutional Review Board, underwent skin prick testing (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 four mold allergens (*Alternaria alternata*, *Aspergillus fumigatus*, *Penicillium* species mix and *Cladosporium* species) (ALK-Abelló Inc.). These symptomatic parents that were also sensitive to at least one aeroallergen were invited to enroll their infant into the CCCAPS cohort.⁵

Clinical visits

At age one, parent(s) brought their infants to CCAAPS for clinical evaluation. The CCAAPS clinical staff interviewed the parent(s) using questionnaires, obtaining details on both the infant's medical history and the home environmental history. The infants were examined and SPT to the same 15 aeroallergens in the parental screening panel in addition to cow's milk and hen's egg. The children returned to the CCAAPS clinics annually at two, three and four years of age for repeat physical examination, skin testing and parental interview. At the year four visit, nasal epithelial smears also were obtained.

Quantitative Skin Prick Testing

Skin prick testing was performed using bifurcated needle coated with the positive histamine dihydrochloride (10 mg/ml) control, the negative 50% glycerinated human serum albumin-saline control, or one of the 17 test panel allergens.⁹ Skin reactions were read after 15 minutes of skin testing. A positive reaction was noted if the diameter was ≥ 3 mm than the negative control in accordance with the most recent the American Academy of Allergy, Asthma and Immunology/and the American College of Allergy, Asthma and Immunology Allergy Diagnostic Practice Parameter.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 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 re-traced and the

enclosed area was calculated using AutoCAD (Autodesk, Inc.). For accuracy, these measurements were performed independently in duplicate by two independent individuals.

Nasal cytology

At age four, each inferior nasal turbinate was swabbed with a separate cotton applicator. The samples were 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 times magnifications until a maximum of 400 cells were counted. For quality control, a second scientist counted 10 percent 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 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 Allergic rhinitis (AR) was defined as a positive response to the ISAAC question, 2) a positive SPT to one of 15 aeroallergens. Allergic eosinophilic rhinitis (AER), the primary outcome of this study, was defined as: 1) a positive response to the ISAAC question, 2) a positive SPT to one of 15 aeroallergens, and 3) greater than 5% nasal eosinophils.³ These AER cases were compared to children without nasal symptoms and negative SPT to all 15 aeroallergens.

Exposure Assessments

Before age one, the CCAAPS research staff visited the infant's home. The home's general characteristics, basement, 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. In order to determine the greatest component of endotoxin, (1-3)- β -D-glucan, and indoor allergen exposure, the infant's primary activity room, a 2 m² area of floor space, was vacuumed at a standard rate of two min/m².^{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 (HDE, endotoxin units (EU)/mg of settled dust) and (1-3)- β -D-glucan (μ g/g of dust) by the limulus amebocyte lysate assay (Associates of Cape Cod Inc.).¹⁷ Separate aliquots of settled dust were used for analysis of major cat allergen (*Fel d1*), major dog allergen (*Can f1*), major dust mite allergen (*Der f1*), and major cockroach allergen (*Bla g1*) by monoclonal sandwich ELISA.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 (NAA), African-American (AA)); gender; annual household income (> \$20,000, \$20,000); breastfeeding duration (months); number of children in the home (2 children, <2 children); season of birth; and aforementioned environmental covariates.²² Hair cotinine levels were measured and used as an objective biomarker of tobacco smoke exposure at age two.²³

Data analysis

The aeroallergen wheal area at ages one, two and three were analyzed for associations to AER using logistic regression. The 95% confidence intervals and odds ratios reported were obtained from the profile likelihood ratio. Any allergen wheal area or covariate significantly associated with AER at the $\alpha < 0.2$ was further evaluated in multivariate logistic regression. Home environmental exposures (endotoxin, β -glucan, *Fel d1*, *Can f1*, *Der p1*, and *Bla g1*) were analyzed as continuous independent variables with thresholds defined by the turning points in the smooth plot that result from the use of general additive model.²⁴ Independent exposures and covariates associated with AER at the 0.2 level were further investigated in 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 age one, two and three regression models was developed. Analyses were performed using SAS 9.2 (SAS Institute Inc.).

Results

Subjects

CCAAPS enrolled 762 infants and 636 (83.5%) were evaluated at age four. Of these, the parents of 478 (75.2%) children consented to nasal eosinophil sampling. Children whose parents did provide consent to nasal sampling were more likely to be NAA, higher father education, higher household income, two children or less in the home and lower elemental carbon attributable to traffic (ECAT) exposure (Table E1 and E2). Of 478 children, 437 (91.4%) children had technically interpretable nasal smears and these were more likely to be from households of higher income, and lower ECAT exposure (Table E3 and E4). Complete rhinitis response, skin testing and interpretable nasal scraping data was available on 434 children.

Sensitivity analyses

To determine if 5% was an acceptable cut-off for the definition of AER, the eosinophil threshold was varied over a range of possible limits. Figure 1 shows 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 the 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 5% or more nasal eosinophils. Of 119 children with 5% or more nasal eosinophils, 79 (66.4%) were sensitized to aeroallergens, while 49 (41.2%) had chronic nasal symptoms. Of the 49 children with 5% or more 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.

Unadjusted analyses

The allergen wheal areas were analyzed for associations with AER after correcting for multiple comparisons. At ages one and two, no allergen wheal area was significantly associated with AER. At age three, *Penicillium* ($p=0.04$), maple ($p=0.02$) and elm ($p=0.11$) wheal area met criteria for further analysis ($p<0.2$) in the multivariable model with AER.

As shown in Table 1, the unadjusted odds ratio of the children characteristics which predicted AER and were included in the multivariable model included gender (OR 2.81; 95% CI, 1.34-6.46; $p=0.009$), mothers' education level (OR 0.37; 95% CI, 0.17-0.76; $p=0.01$), breastfeeding duration (OR 0.55; 95% CI, 0.27-1.08; $p=0.09$). The unadjusted odds ratio of environmental exposures meeting the inclusion criteria were only low, medium and high house dust *Fel d1* levels (Table 2). ECAT showed a non-significant protective association. After further investigation, it was determined that a positive SPT at ages two and three were acting as intervening variable, thus necessitating removal from further analysis.

Adjusted analysis

Table 3 shows the adjusted odds ratio of allergen wheal area and covariates (children characteristics and environmental exposures). At age three, *Penicillium* (aOR 1.18; 95% CI, 1.06-1.32; $p=0.002$) and maple (aOR 1.07; 95% CI, 1.01-1.13; $p=0.02$) 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; $p=0.11$). In comparison to the binary (positive or negative) measure of aeroallergens, the *Penicillium* wheal area was more precise (smaller confidence intervals) and more significantly associated with AER. The two tree measures also were more precise (smaller confidence intervals) compared to the binary values but the significance levels were comparable.

These three informative allergen wheal areas at age three were summed and showed a significant linear relationship to AER (OR 1.1; 95% CI, 1.02-1.09; $p=0.003$). These summed wheal areas were compared to children that were negative for all three allergens. As shown in Figure 2, each percentile showed a dose dependent increased risk of AER. Children with a sum of all three allergen wheal areas in the 25th percentile, in the > 25th and < 75th and in the 75th percentile had an increased risk of AER (OR 3.6; 95% CI, 1.12-10.47; $p=0.03$), (OR 4.1; 95% CI, 1.78-9.01; $p=0.0001$) and (OR 10.7; 95% CI, 3.43-34.20; $p<0.0001$) respectively. Male gender (OR 2.5 95% CI, 1.07-6.44; $p=0.04$) and higher mother's education (OR 0.38; 95% CI, 0.16-0.87; $p=0.03$) were also significantly associated with AER.

Discussion

Previously, little has been reported about the etiology and significance of nasal eosinophilia in children. In a small study of twenty adolescent Italian children with perennial AR, nasal eosinophils were significantly associated with 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 Our study is the first to demonstrate that early aeroallergen sensitization is associated with children with AR that also have a high number of nasal eosinophils (AER) at age four in a large North American cohort. Of the 434 children in this study, 38 (8.8%) had AER.

The present study is 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 as early as age three, aeroallergen wheal areas are significantly associated to AER at age four. Our results indicate that especially *Penicillium* and maple wheal areas (with elm wheal of borderline significance) at age three are most predictive of AER. Those children with the largest sum of these three informative wheal areas had an approximate 11-fold increase in risk of AER. At ages one and two, however, after controlling for relevant covariates, no significant associations were observed with allergen wheal areas and AER at age four, indicating that in this cohort earlier aeroallergen sensitization is less informative for AER at age four. Our group has previously reported that *Penicillium* is the most prevalent measureable fungal species in both the indoor and outdoor environment.²⁷ Recent evidence suggests that *Penicillium* is important to allergic respiratory disorders. In a murine model of asthma, *Penicillium* extract induced more vigorous inflammatory response in bronchoalveolar lavage fluid compared to house dust mite extract.²⁸ In Puerto Rican inner-city children, *Penicillium* mold count in the child's bedroom was associated with increased frequency of asthma symptoms although no tests of sensitization were performed.²⁹ Another study revealed that *Penicillium* sensitization increases with age among asthmatic children.³⁰ More recent studies have demonstrated that the presence of *Pencillium* in the air was associated with infant wheezing.³¹ In school age asthmatic children, *Penicillium* sensitization and exposure increased the risk of wheezing and asthma severity score.³² *Penicillium* sensitization frequency in our cohort also increased at ages one (n=8), two (n=12) and three (n=25) respectively. Less is known regarding 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 were correlated with asthma hospital admissions in Canada, and levels were positively correlated with asthma hospital admissions in Portugal.^{34,35} We showed in previous work that tree sensitivity at age one was associated with AR at age three.²² Our 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 skin testing and use of eosinophils to measure AR severity. The nasal eosinophils provide an objective biomarker of a more specific phenotype of AER. This phenotype may be useful as a severe phenotype for future intervention trials especially for high risk children i.e. those born to atopic parents. The large sample size at age four allowed for investigation of less prevalent outcomes, such as AER. The use of wheal areas as a continuous measurement increased the power to detect association to AER.

As with all studies, there were limitations. The children were skin tested to relevant aeroallergens for the Greater Cincinnati metropolitan area and therefore may limit the generalizability to other regions. The importance of different sets of aeroallergen skin test results in other regions will need to be determined. Children whose parents consented to nasal scraping were more often NAA. However, when analyzing if 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 earlier ages. Therefore no assumptions can be made regarding if 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 age four AER. None were predictive prior to age three, but *Penicillium* and maple wheal areas at age three were significantly associated with AER at age four. Hence, at least as early as three years old may be an important time to begin SPT children especially from high risk families. Children with the largest sum of wheal areas for *Penicillium*, maple and elm at age three were at greatest risk of AER at age four. 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.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

We would like to thank Stacy Langemeyer, BS who contributed in the collection, quantifying and storing the nasal eosinophils.

Supported by: National Institute of Environmental Health Services Grant ES 11170 and ES 10957; National Institute of Allergy and Infectious Disease AI 60515 T32

References

1. Stone KD, Prussin C, Metcalfe DD. IgE, mast cells, basophils, and eosinophils. *The Journal of Allergy and Clinical Immunology*. Feb; 2010 125(2 Suppl 2):S73–80. [PubMed: 20176269]
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*. Mar; 2005 136(3):266–272. [PubMed: 15722636]
3. Amorim MM, Araruna A, Caetano LB, Cruz AC, Santoro LL, Fernandes AL. Nasal eosinophilia: an indicator of eosinophilic inflammation in asthma. *Clinical and experimental allergy : journal of the British Society for Allergy and Clinical Immunology*. Jun; 2010 40(6):867–874. [PubMed: 20100189]
4. Malmberg H. Symptoms of chronic and allergic rhinitis and occurrence of nasal secretion granulocytes in university students, school children and infants. *Allergy*. Dec; 1979 34(6):389–394. [PubMed: 546254]
5. LeMasters GK, Wilson K, Levin L, et al. High prevalence of aeroallergen sensitization among infants of atopic parents. *J Pediatr*. Oct; 2006 149(4):505–511. [PubMed: 17011322]
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*. Aug; 2005 116(2):279–284. [PubMed: 16083780]

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*. Dec 1; 2009 180(11):1068–1075. [PubMed: 19745206]
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). *The Journal of Allergy and Clinical Immunology*. Feb.2013 131(2 Supplement):AB237.
9. Bernstein IL, Li JT, Bernstein DI, et al. Allergy diagnostic testing: an updated practice parameter. *Ann Allergy Asthma Immunol*. Mar; 2008 100(3 Suppl 3):S1–148.
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*. May; 2004 134(1):34–40. [PubMed: 15051938]
11. Ciprandi G, Tosca M, Ricca V, et al. Cetirizine treatment of allergic cough in children with pollen allergy. *Allergy*. Jul; 1997 52(7):752–754. [PubMed: 9265991]
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-naïve children. *Ann Allergy Asthma Immunol*. Jun; 2003 90(6):640–645. [PubMed: 12839323]
13. Asher MI, Keil U, Anderson HR, et al. International Study of Asthma and Allergies in Childhood (ISAAC): rationale and methods. *Eur Respir J*. Mar; 1995 8(3):483–491. [PubMed: 7789502]
14. Iossifova YY, Reponen T, Bernstein DI, et al. House dust (1-3)-beta-D-glucan and wheezing in infants. *Allergy*. May; 2007 62(5):504–513. [PubMed: 17441791]
15. Hamilton RG. Assessment of indoor allergen exposure. *Curr Allergy Asthma Rep*. Sep; 2005 5(5):394–401. [PubMed: 16091213]
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. *Annals of allergy, asthma & immunology : official publication of the American College of Allergy, Asthma, & Immunology*. Aug; 2011 107(2):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*. Dec; 1997 58(12):861–867. [PubMed: 9425646]
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*. Feb 1; 1988 140(3):812–818. [PubMed: 3276780]
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*. Aug; 1987 80(2):184–194. [PubMed: 3611539]
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*. Feb; 1991 87(2):505–510. [PubMed: 1993810]
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*. Dec; 1991 88(6):847–853. [PubMed: 1744355]
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*. May; 2010 125(5):1054–1060 e1051. [PubMed: 20392478]
23. Biagini Myers JM, Hershey GK, Deka R, et al. Asking the Right Questions to Ascertain Early Childhood Secondhand Smoke Exposures. *The Journal of Pediatrics*. 2012
24. Harrell, J.; Frank, E. Regression modeling strategies: with application to linear models, logistic regression, and survival analysis. New York: Springer-Verlag New York Inc.; 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*. Jul; 2005 60(7):957–960. [PubMed: 15932388]

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. *Journal of microbiology, immunology, and infection = Wei mian yu gan ran za zhi*. Jun; 2006 39(3):212–218.
27. Osborne M, Reponen T, Adhikari A, et al. Specific fungal exposures, allergic sensitization, and rhinitis in infants. *Pediatr Allergy Immunol*. Sep; 2006 17(6):450–457. [PubMed: 16925691]
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*. Oct; 2010 20(5):380–391. [PubMed: 20590919]
29. Turyk M, Curtis L, Scheff P, et al. Environmental allergens and asthma morbidity in low-income children. *J Asthma*. Aug; 2006 43(6):453–457. [PubMed: 16952864]
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. *Annals of allergy, asthma & immunology : official publication of the American College of Allergy, Asthma, & Immunology*. Feb; 2003 90(2):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*. Nov; 2013 68(11):1410–1418. [PubMed: 24118031]
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*. Oct.2012 118:86–93. [PubMed: 22863552]
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*. Feb; 2005 94(2):240–246. [PubMed: 15765739]
34. Dales RE, Cakmak S, Judek S, Coates F. Tree pollen and hospitalization for asthma in urban Canada. *Int Arch Allergy Immunol*. 2008; 146(3):241–247. [PubMed: 18270491]
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*. Apr; 2009 109(3):328–333. [PubMed: 19147130]

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

Figure 2. Odds ratios of developing allergic eosinophilic rhinitis at age four by percentiles of total of important allergen wheal areas* from multivariate regression model at age three
*Allergen wheal areas that were included in the final multivariate model at age 3 includes: *Penicillium*, Maple, and Elm.

Table 1
Unadjusted children characteristics to Allergic Rhinitis with high eosinophils (AER)
compared to the combined phenotypes other than AR at age four

A total of 434 children had complete questionnaire, SPT and nasal cytology at age four.

Covariates	Frequency (%)	Allergic Eosinophilic Rhinitis (AER) n=38/434 (8.8)	All phenotypes other than AER n=335/434 (77.2)	OR (95% CI)
Gender:				
Female	193 (44.5)	9 (4.7)	156 (80.8)	2.81
Male	241 (55.5)	29 (12.0)	179 (74.3)	(1.34, 6.46)[†]
Race:				
NAA	345 (79.5)	31 (9.0)	268 (77.7)	0.90
AA	89 (20.5)	7 (7.9)	67 (75.3)	(0.35, 2.03)
Mother's Education:				
College graduate	213 (50.7)	27 (12.7)	161 (75.6)	0.37
Some college/trade	207 (49.3)	10 (4.8)	162 (78.3)	(0.17, 0.76)[‡]
Father's Education				
Some college/trade	298 (71.1)	29 (9.7)	232 (77.9)	0.71;
HS diploma or less	121 (28.9)	8 (6.6)	90 (74.4)	(0.29, 1.55)
Household Income:				
\$20 K	354 (84.7)	33 (9.3)	273 (77.1)	0.69
< \$20 K	64 (15.3)	4 (6.3)	48 (75.0)	(0.20, 1.83)
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 (months):				
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)[*]

^{*} $P < 0.2$

[†] $P < 0.05$

[‡] $P < 0.003$

Table 2
Unadjusted environmental exposure association to Allergic Rhinitis with high eosinophils (AER) compared to the combined phenotypes other than AR at age four

A total of 434 children had complete questionnaire, SPT and nasal cytology at age four. Not shown are the allergic rhinitis without high nasal eosinophils (n=61).

Environmental Exposure	Number (%)	Allergic Eosinophilic Rhinitis (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)	N.C.
230-640	39 (9.0)	6 (15.4)	29 (74.4)	N.C.
640	7 (1.6)	0 (0)	6 (85.7)	N.C.
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 d1 (μg/ml) <4.1	76 (17.5)	3 (4.0)	65 (85.5)	3.38;(0.95,18.67)*
4.1-148.4	230 (53.0)	20 (8.7)	179 (77.8)	0.24; (0.04, 1.09)*
148.4	128 (29.5)	15 (11.7)	91 (71.1)	1.64; (0.92 2.93)*
Der p1 (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 f1 (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 g1 (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)	N.C.
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)*
Children in home at 12 months				
2 child	141 (32.5)	9 (6.4)	113 (80.1)	1.64
< 2 child	293 (67.5)	29 (9.9)	222 (75.8)	(0.78, 3.78)
Stays in daycare-like facility for 8 hours during 1st year.				

Environmental Exposure	Number (%)	Allergic Eosinophilic Rhinitis (AER) n=38/434 (8.8)	All phenotypes other than AER n=335/434 (77.2)	OR (95% CI)
No	277 (64.9)	28 (10.1)	214 (77.3)	0.53
Yes	150 (35.1)	8 (5.3)	116 (77.3)	(0.22, 1.14)
No. colds at 12 months:				
< 7 colds	396 (91.2)	35 (8.8)	307 (77.5)	0.94
7 colds	38 (8.8)	3 (7.9)	28 (73.7)	(0.22, 2.83)

*
P<0.2

Table 3
Adjusted odds ratio (aOR) and 95% confidence intervals of allergen wheal area at each year associated to allergic eosinophilic rhinitis at age four

Gender and mother's education were included in age three model to improve model fit.

		Age Three	
Allergens	Elm	Allergen Wheal Area aOR; 95% CI; <i>P</i> -value	Allergen Binary value aOR; 95% CI; <i>P</i> -value
	Elm	1.06; (0.98, 1.14); 0.11	2.93; (0.68, 10.92); 0.12
	Maple	1.07; (1.01, 1.13); 0.02	3.72; (1.11, 11.53); 0.03
	Penicillium	1.18; (1.06, 1.32); 0.002	4.29; (0.99, 15.86); 0.03