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Traffic pollution is associated with early childhood aeroallergen sensitization

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A R T I C L E I N F O

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A B S T R A C T

Background: No large, prospective, epidemiologic study has investigated the association between diesel exhaust particle (DEP) exposure and early aeroallergen sensitization and allergic rhinitis (AR) at 4 years of age.

Objective: To determine how exposure to traffic exhaust during infancy is associated with aeroallergen sensitization and AR at 4 years of age and the predictive utility of the wheal area at 1 to 3 years of age on AR at 4 years of age.

Methods: Infants born to aeroallergen sensitized parents were evaluated annually with skin prick tests to 15 aeroallergens with measurement of wheal areas. At 4 years of age, AR was defined as at least one positive aeroallergen skin prick test result and the presence of sneezing and a runny nose without a cold or flu. Infant (DEP) exposure was estimated using data from 27 air sampling monitors and a land use regression model.

Results: Complete data were available for 634 children at 4 years of age. Prevalence of AR increased annually from 6.9% to 21.9%. A positive trend was observed for high DEP exposure and aeroallergen sensitization at 2 and 3 years of age (odds ratio, 1.40; 95% confidence interval, 0.97–2.0) and (odds ratio, 1.35; 95% confidence interval, 0.98–1.85) but not with AR. At 2 years of age, every 1-mm^2 increase in the wheal area of timothy and *Alternaria* significantly increased the odds of AR at 4 years of age. At 3 years of age, every 1-mm^2 increase in the wheal area of fescue, dog, and *Penicillium* significantly increased the odds of AR at 4 years of age.

Conclusion: DEP exposure enhances the risk of early aeroallergen sensitization. Aeroallergen wheal area at 2 and 3 years of age is associated with AR at 4 years of age.

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Introduction

Allergic rhinitis (AR) is an IgE-mediated disease with symptoms of rhinorrhea, nasal obstruction, nasal itching, and sneezing on exposure to aeroallergens. In the United States, approximately $11.2 billion was spent on AR treatments in 2005.[1] Preschool children and young adults with AR are at increased risk of asthma later in life.[2,3] Early exposure to traffic-related air pollutants, specifically diesel exhaust particles (DEPs), may enhance the risk of aeroallergen sensitization and development of allergic disorders in childhood.[4,5] No prospective study has defined the exact association between early DEP exposure and percutaneous aeroallergen sensitization, especially the predictive value of early sensitization, during the first 3 years after birth and the subsequent development of childhood AR. To address these gaps, we conducted the Cincinnati Childhood Allergy and Air Pollution Study (CCAAPS) to test the hypothesis that childhood DEP exposure is associated with development of aeroallergen sensitization and allergic disease.[6] In addition, we examined the predictive utility aeroallergen wheal area on AR at 4 years of age.

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Table 1
Characteristics of children from the cincinnati childhood allergy and air pollution study

Covariate	Total, No. (%)	AR, No. (%) (n = 139)	No or phenotypes other than AR, No. (%) (n = 495)	OR (95% CI)
Sex				
Female	290 (45.7)	58 (20.0)	232 (80.0)	1 [Reference]
Male	344 (54.3)	81 (23.6)	263 (76.5)	1.23 (0.84–1.80)
Ethnicity				
Non–African American	492 (77.6)	102 (20.7)	390 (79.3)	1 [Reference]
African American	142 (22.4)	37 (26.1)	105 (73.9)	1.35 (0.87–2.07)[a]
Mother's educational level				
College graduate	307 (50.0)	74 (24.1)	233 (75.9)	1 [Reference]
Some college or trade school	307 (50.0)	63 (20.5)	244 (79.5)	0.81 (0.55–1.19)
Father's educational level				
Some college or trade school	425 (69.3)	93 (21.9)	332 (78.1)	1 [Reference]
High school diploma or less	188 (30.7)	44 (23.4)	144 (76.6)	1.09 (0.72–1.63)
Household income				
≥$20,000	502 (82.3)	109 (21.7)	393 (78.3)	1 [Reference]
<$20,000	108 (17.7)	28 (25.9)	80 (74.1)	1.26 (0.77–2.02)
Season of birth				
Winter	209 (33.0)	45 (21.5)	164 (78.5)	1 [Reference]
Spring	140 (22.1)	29 (20.7)	111 (79.3)	0.95 (0.56–1.60)
Summer	138 (21.8)	32 (23.2)	106 (76.8)	1.10 (0.65- 1.84)
Autumn	147 (23.2)	33 (22.5)	114 (77.6)	1.06 (0.63–1.75)
Breastfeeding duration, mo				
≥4	289 (45.6)	66 (22.8)	223 (77.2)	1 [Reference]
<4	345 (54.4)	73 (21.2)	272 (78.8)	0.91 (0.62–1.32)
Children in the home at 12 mo				
≥2	197 (31.1)	36 (18.3)	161 (81.7)	1 [Reference]
<2	437 (68.9)	103 (23.6)	334 (76.4)	1.38 (0.91–2.13)[a]
Stays in daycare-like facility for ≥8 hours during first year				
No	399 (64.2)	89 (22.3)	310 (77.7)	1 [Reference]
Yes	223 (35.9)	48 (21.5)	175 (78.5)	0.96 (0.64–1.42)
No. colds reported at 1 year of age				
<7 colds	581 (91.6)	125 (21.5)	456 (78.5)	1 [Reference]
≥7 colds	53 (8.4)	14 (26.4)	39 (73.6)	1.31 (0.67–2.44)

Abbreviations: AR, allergic rhinitis; CI, confidence interval; OR, odds ratio.
Baseline comparison categories are indicated first, followed by categories associated with increased risk of AR. Because of incomplete parental responses, the sum of response to each covariable does not always total 634.
[a] $P < .20$.

Percutaneous skin prick tests (SPTs) are the most practical method for determining allergen specific IgE-mediated mast cell degranulation, especially in young children.[7] The importance of quantifying the SPT response is widely appreciated.[8] The SPT wheal diameter predicts probability of a positive controlled food challenge test in patients with a history of food allergy and may reduce the need for oral food challenges compared with in vitro IgE testing.[9,10] Asymptomatic birch-sensitized adults with a wheal diameter area of 4 mm or greater are at increased risk for later development of clinical allergy (nasal, ocular, or respiratory symptoms).[11] Positive SPT results to outdoor allergens in adolescence is associated with persistent AR.[12] Cat sensitization is associated with asthma and hay fever in young adults.[13] Our second hypothesis is SPT wheal area during the first 3 years of childhood is a continuous predictor of the future development of AR at 4 years of age.

Methods

Study Design and Location

The CCAAPS was approved by the University of Cincinnati Institutional Review Board. Infants born within the Greater Cincinnati/Northern Kentucky area were identified from birth records. Infants living less than 400 m or greater than 1,500 m from a major road (>1,000 trucks traveling per day) were eligible to participate. Parents were informed of the study and signed an informed consent form approved by the University of Cincinnati Institutional Review Board.[14] If at least one parent reported naso-ocular symptoms and/or dyspnea on exposure to pollens, animals, bedrooms, or exercise, they underwent skin prick testing to 15 regionally relevant aeroallergens, including white oak, American elm, maple mix, eastern red cedar, meadow fescue, timothy, short ragweed, cat, dog, house dust mite mix (*Dermatophagoides farinae and Dermatophagoides pteronyssinus*), German cockroach, and 4 mold allergens (*Alternaria alternata, Aspergillus fumigatus, Penicillium* species mix, and *Cladosporium* species) (ALK-Abelló Inc).[14] Infants of these symptomatic and SPT-positive parents were enrolled during the first year after birth and constituted the ongoing CCAAPS cohort.

Clinical Visits

Eligible children were enrolled, and beginning at 1 year of age, trained clinical staff administered questionnaires to parents regarding the child's medical history during the previous year and a home environmental history questionnaire. Children were examined and skin tested to the same 15 regional aeroallergens used in parental screening and cow's milk and hen's egg (ALK-Abelló Inc). The questionnaires, physical examination, and SPTs were performed annually until 4 years of age.

Health Outcomes

At the clinical visits, parents were queried about the presence of rhinitis symptoms using the International Study of Asthma and Allergies in Childhood 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?"[15,16] One of the primary CCAAPS outcome, AR at 4 years of age, was defined as a positive parental response to the International Study of Asthma and Allergies in Childhood question and a positive SPT result to 1 or more of 15 aeroallergens at 4 years of age. Aeroallergen

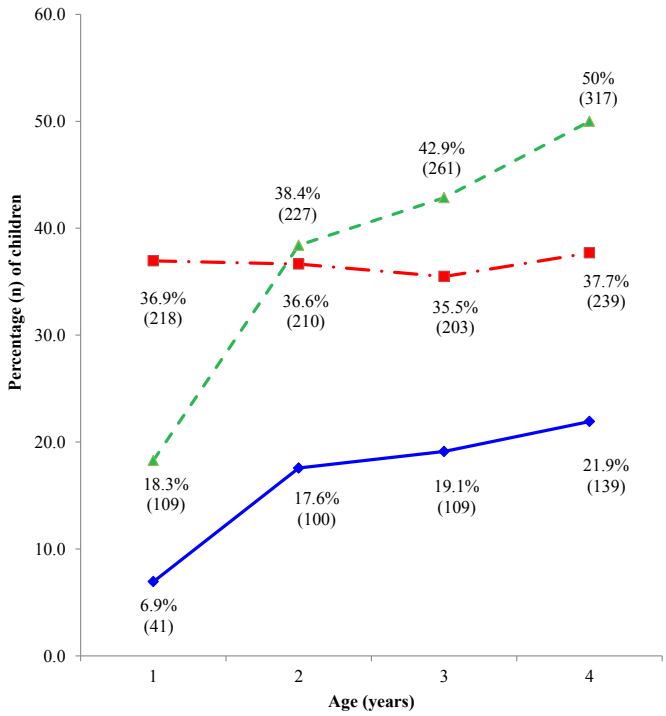

Figure 1. Prevalence of allergic rhinitis, aeroallergen sensitization, and rhinitis during the first 4 years after birth among the 634 study participants who had complete clinical and skin test data at 4 years of age. Allergic rhinitis prevalence increased during the 4 years of the study. Although rhinitis symptoms remained stable during the study, the prevalence of aeroallergen sensitization increased. Blue line indicates allergic rhinitis; red line, rhinitis; and green line, aeroallergen sensitization.

sensitization at all ages was defined as a positive SPT result to 1 or more of 15 aeroallergens. Children who did not have AR (including non-AR, atopic but asymptomatic and nonatopic, asymptomatic children) served as comparison group for this study.

Quantitative SPTs

At annual visits, SPTs were performed using bifurcated needle (Duo-tip, Lincoln Diagnostics) with a drop of allergen extract (ALK-Abelló Inc). Histamine dihydrochloride (10 mg/mL) and 50% glycerinated human serum albumin-saline control were used as positive and negative controls, respectively (Hollister-Stier Laboratories LLC). The SPT results were interpreted after 15 minutes, with a positive SPT result defined as a wheal of 3 mm greater than the negative saline control. The circumferences of all positive wheal reactions to histamine, saline, and each allergen were traced with a pen. The ink tracing was transferred to labeled grid paper to create a permanent record and scanned as true image files for analysis in AutoCAD (Autodesk Inc). The ink circumferences of the wheal reactions were traced in AutoCAD, allowing calculation of the enclosed area by planimetry. The wheal area was used as a continuous independent variable.

Traffic Exposure Estimation

The method of estimation of individual exposure to DEP has been previously published.[17–19] Harvard-type Impactors (Air Diagnostics and Engineering) were used to collect fine particulate matter (diameter $\leq$2.5 mm [$PM_{2.5}$]) on 37-mm membrane Teflon filters (nominal pore size, 1 μm) and 37-mm quartz filters. The elemental carbon concentration of $PM_{2.5}$ was determined from the quartz filters (via the thermal optical transmittance technique) and the Teflon filters (via the reflectance technique). Using the optical transmittance technique, the quartz filters were used to determine the organic carbon concentrations of $PM_{2.5}$. Teflon filters were also analyzed for $PM_{2.5}$ mass by gravimetric analysis and for 38 elements using x-ray fluorescence to develop a diesel signature profile.[18,20] The elemental carbon attributable to traffic was determined by using the multivariable receptor model UNMIX and by the chemical mass balance model. This elemental carbon attributable to traffic was used as an estimate of DEP exposure. The estimate for DEP was further validated with additional analysis identifying subfractions of elemental and organic carbon.[19] DEP exposure was estimated using a land-use regression model that included wind direction, elevation, length of bus route, and truck intensity within 300 m of sampling site and applied to all locations where the child spent 8 or more hours during their first year of life.[21]

Indoor Home Assessments and Measurements of Allergens and Endotoxin

Before 1 year of age, the CCAAPS infant's home was visited by trained research staff.[22] Each room in the home was evaluated for visible mold, water damage, and general state of repair. Settled house dust was collected from the infant's primary activity room and analyzed for endotoxin, β-glucan, Fel d 1 (cat), Can f 1 (dog), Der p 1 (house dust mite), and Bla g 1 (cockroach) allergens as described previously.[23–28]

Covariates

The CCAAPS had previously identified risk factors of AR at 1 year and 3 years of age, including ethnicity (non–African American vs African American), sex (female vs male), annual household income (>$20,000 vs $\leq$$20,000), breastfeeding duration (months), number of children in the home ($\geq$2 children vs <2 children), and season of birth.[26] Hair cotinine levels at 2 years of age were measured and included in this study as an objective marker of secondhand smoke exposure during early childhood.[29] These covariates were evaluated along with DEP, endotoxin, and indoor allergen levels.

Statistical Analysis

A detailed description and rationale of the statistical plan are provided online (eMethods). Before bivariate comparison, continuous exposure covariates (endotoxin, β-glucan, Fel d 1, Can f 1, Der p 1, and Bla g 1) were analyzed by a general additive model to show turning points (ie, changes in direction) in the smooth plot, indicating changes from a linear category threshold.[30] High DEP was defined as exposure above the 66th percentile as previously described.[28] Logistic regression was performed to determine whether the 17 allergen wheal areas at 1, 2, and 3 years of age were associated with AR at 4 years of age, after adjusting for multiple testing using the Holms-Sidak test. Logistic regression was used to determine associations between the covariates and AR at 4 years of age. Any independent predictor variable associated with AR ($P <$ .20) was further investigated in a multivariable age-stratified model.

Each age-specific multivariable model was reduced by the "all subsets" method of selection to include variables that improved model fit.[31] The final simplified regression model was achieved by removing an independent variable if the log likelihood ratio did not decrease significantly and/or the remaining variable coefficients did not change by more than 20%. Additional stratified analyses were performed using same method. To confirm the findings, any allergen wheal area that improved the final age-stratified regression model fit was summed and then analyzed for associations to AR. All analyses were performed using SAS statistical software, version 9.3 (SAS Institute Inc).

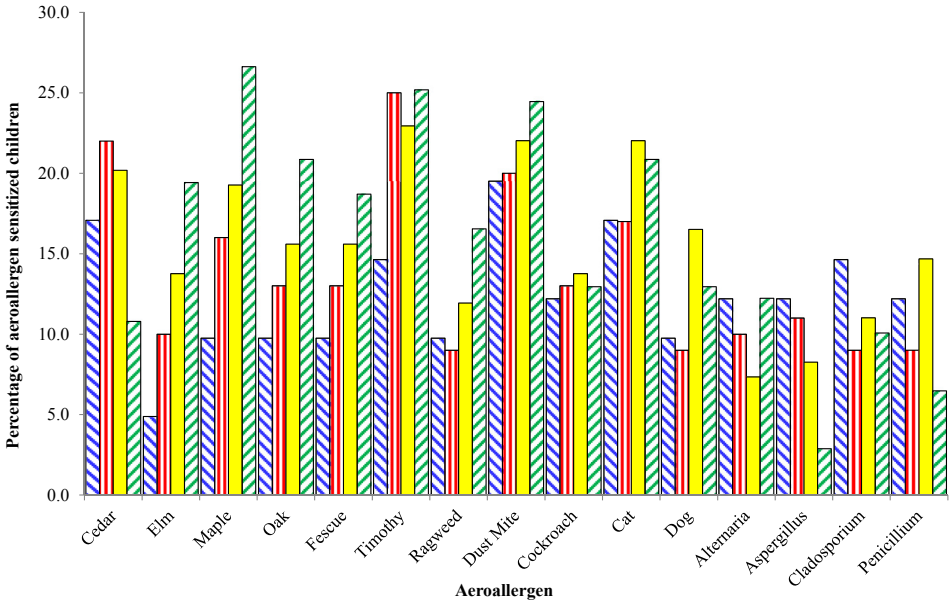

Figure 2. Percentage of aeroallergen sensitivities among allergic rhinitis children at 1 to 4 years of age. Blue striped bar indicates year 1; red striped bar, year 2; yellow bar, year 3; and green striped bar, year 4.

Results

Study Participants

Seven hundred sixty-two children were enrolled in the CCAAPS. Of these, 638 children returned for clinical evaluation at 4 years of age; rhinitis symptom and skin testing data were available for 634 children (83%). Table 1 gives the demographic characteristics of children in the study and associations to AR. Only ethnicity ($P = .18$) and the presence of 2 or more children in the home during year 1 ($P = .14$) met criteria for evaluation in the multivariate model. Because of previous associations with AR at 3 years of age in this cohort, breastfeeding duration and season of birth were examined in the multivariable model.[26]

The prevalence estimates of AR, aeroallergen sensitization, and rhinitis are shown in Figure 1. The prevalence of AR increased annually from 7.0% at 1 year of age to 21.9% at 4 years of age. When looking at the components of AR, aeroallergen sensitization prevalence increased annually from 18.3% at 1 year of age to 50% at 4 years of age, whereas the prevalence of rhinitis symptoms remained relatively constant.

The percentage and absolute number of AR children sensitized to each aeroallergen at each age is shown in Figure 2 and eFigure 1, respectively. Timothy was the most prevalent positive SPT response at 2 years of age (25%) and 3 years of age (22.9%) and second (25.2%) only to maple (26.6%) at 4 years of age. Dust mite and cat were the most common indoor aeroallergen sensitizations in all 4 years. To determine how the magnitudes of these sensitivities at each year are associated with AR at 4 years of age, the wheal areas were examined as continuous measurements.

Unadjusted Analyses

The number of allergens associated with AR ($P < .20$ for the multivariate model inclusion) increased annually, from none in year 1 to 4 (maple, timothy, cat, and *Alternaria*) and 6 (elm, maple, fescue, timothy, dog, and *Penicillium*) allergens in years 2 and 3, respectively (eTable 1). The environmental exposure covariates measured at 1 year of age and their unadjusted associations with AR at 4 years of age are given in Table 2. The factors from the settled house dust samples that met inclusion criteria included low ($P = .10$), medium ($P = .04$), and high ($P = .11$) levels of endotoxin; medium ($P = .17$) and high ($P = .17$) β-glucan; and low, medium, and high Fel d 1 exposure ($P = .03$ each). Low levels of hair cotinine at 2 years of age was not significantly associated with AR at 4 years of age ($P = .16$).

High DEP exposure at 1 year of age had a protective effect for AR at 4 years of age (Table 2, $P = .09$). This finding led us to examine the association between DEP and the other covariates. Further analysis revealed that DEP was borderline associated with aeroallergen sensitization at 2 (OR 1.40; 95% CI [0.97, 2.00]; 0.07) and 3 years of age (OR = 1.35; 95% CI [0.98,1.85]; 0.07) (vertical black arrows, Fig 3B and C). Sensitization at 1, 2, and 3 years of age was significantly associated with AR at 4 years of age (downward diagonal black arrow, Fig 3A–C). Because DEP is related to aeroallergen sensitization (the main risk factor of interest in this study), the aeroallergen sensitization is an intermediate state in the development of AR. Therefore, DEP was removed from the adjusted multivariate analysis.

Adjusted Analyses

Table 3 gives the results of multivariable regression at each age. At 1 year of age, none of the 15 aeroallergen wheal areas were significantly associated with AR at 4 years of age. At 2 years of age, the final model included the following aeroallergen wheal areas: timothy (adjusted odds ratio [aOR], 1.06; 95% confidence interval [CI], 1.02–1.11; $P = 0.01$) and *Alternaria* (aOR, 1.07; 95% CI, 1.00–1.15; $P = .04$). The covariates in the final multivariable model at 2 years of age included breastfeeding duration, Fel d 1, and season of birth. At 3 years of age, the following aeroallergen wheal areas were included: maple (aOR, 1.04; 95% CI, 1.00–1.09; $P = .08$), fescue (aOR, 1.08; 95% CI, 1.02–1.15; $P = .02$), dog (aOR, 1.06; 95% CI, 1.01–1.12; $P = .03$), and *Penicillium* (aOR, 1.14; 95% CI, 1.06–1.23; $P = .001$). The covariates in the final multivariable model at 3 years of age included ethnicity and number of children in the home at 1 year of age.

All the informative allergen wheal areas ($P < .20$) at 2 and 3 years of age were included into a single regression model. The wheal areas for *Alternaria* at 2 years of age (OR, 1.11; 95% CI, 1.03–1.21; $P = .01$), maple at 3 years of age (OR, 1.05; 95% CI, 1.00–1.10; $P = .047$), fescue at 3 years of age (OR, 1.08; 95% CI,

Table 2
Frequency and unadjusted ORs (95% CIs) of covariates and their association with AR[a]

Covariate	Total, No. (%)	AR, No. (%) (n = 139)	No or phenotypes other than AR (n = 495)	OR (95% CI)
Endotoxin, EU/mg of dust				
<230	567 (89.4)	124 (21.9)	443 (78.1)	1.00 (1.00–1.01)[a]
230–640	57 (9.0)	13 (22.8)	44 (77.2)	0.99 (0.98–1.00)[b]
≥640	10 (1.6)	2 (20.0)	8 (80.0)	1.02 (1.00–1.05)[a]
β-glucan, mg/g of dust				
<60	401 (63.3)	90 (22.4)	311 (77.6)	1.01 (1.0–1.02)
60–170	159 (25.1)	35 (22.0)	124 (78.0)	0.99 (0.97–1.01)[a]
≥33.12	74 (11.7)	14 (18.9)	60 (81.1)	1.01 (1.0–1.02)[a]
Fel d 1, mg/mL				
<4.1	113 (17.8)	17 (15.0)	96 (85.0)	1.97 (1.07–3.79)[b]
4.1–148.4	337 (53.2)	75 (22.3)	262 (77.7)	0.42 (0.19–0.90)[b]
≥148.4	184 (29.0)	47 (25.5)	137 (74.5)	1.48 (1.05–2.08)[b]
Der p 1, mg/mL				
<54.6	485 (76.5)	106 (21.9)	379 (78.1)	1.07 (0.92–1.24)
≥54.6	149 (23.5)	33 (22.2)	116 (77.9)	0.79 (0.53–1.16)
Can f 1, mg/mL				
<0.74	295 (46.5)	67 (22.7)	228 (77.3)	0.99 (0.06–20.56)
0.74–9.03	147 (23.2)	33 (22.5)	114 (77.6)	1.02 (0.07–12.56)
9.03–221.4	149 (23.5)	31 (20.8)	118 (79.2)	0.93 (0.49–1.79)
≥221.4	43 (6.8)	8 (18.6)	35 (81.4)	1.07 (0.69–1.65)
Bla g 1, mg/mL				
<0.07	612 (96.5)	133 (21.7)	479 (78.3)	0.96 (0.62–1.46)
≥0.07	22 (3.5)	6 (27.3)	16 (72.7)	1.14 (0.86–1.56)
Cotinine at 2 years of age, ng/mg of hair				
<0.11	447 (70.5)	94 (21.0)	353 (79.0)	1.39 (0.88–2.20)[a]
0.11–0.67	160 (25.2)	37 (23.1)	123 (76.9)	0.87 (0.66–1.13)
≥0.67	27 (4.23)	8 (29.6)	19 (70.4)	
DEP exposure at 1 year of age, mg/m^3				
≤0.32	423 (66.7)	101 (23.9)	322 (76.1)	1 [Reference]
>0.32	211 (33.3)	38 (18.0)	173 (82.0)	0.70 (0.46–1.05)[a]

Abbreviations: AR, allergic rhinitis; CI, confidence interval; OR, odds ratio.
Baseline comparison categories are indicated first, followed by categories associated with increased risk of AR. Because incomplete parental responses, the sum of response to each covariable do not always total 634.
[a]$P < .20$.
[b]$P < .05$.

1.02–1.15; $P = .03$), dog at 3 years of age (OR, 1.06; 95% CI, 1.01–1.12; $P = .08$), and *Penicillium* at 3 years of age (OR, 1.14; 95% CI, 1.06–1.23; $P = .002$) were significantly associated with AR at 4 years of age (Table 3).

We further investigated 2 and 3 years of age to determine whether the wheal area association differed among the strata of children who were aeroallergen sensitized but asymptomatic or among those with early AR (eTable 2 and eTable 3, respectively). Among aeroallergen sensitized but asymptomatic children, elm wheal area at 3 years of age met the criteria ($P < .2$) for further analysis ($P = .10$; eTable 2). This effect was significant in a multivariable model (aOR, 1.08; 95% CI, 1.03–1.21; $P = .01$; eTable 4). Among children with early AR, *Alternaria* wheal area at 2 years of age met criteria ($P < .2$) for further analysis ($P = .002$; eTable 3), and this gained significance in a multivariable model (aOR, 1.19; 95% CI, 1.05–1.48; $P = .03$; eTable 4).

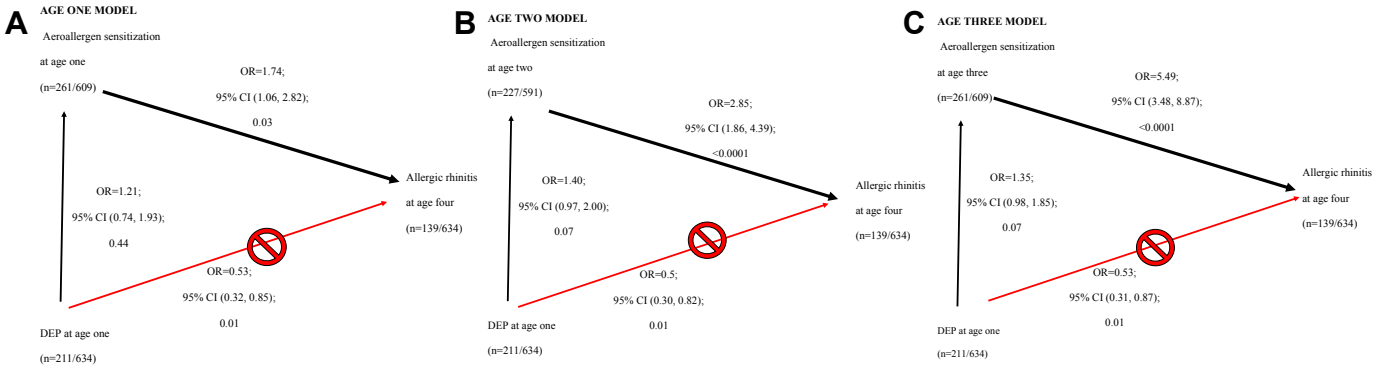

Figure 3. Directed acyclic graph showing how diesel exhaust particle (DEP) exposure at 1 year of age (≥66th vs <66th percentile) confounds aeroallergen sensitization at 1 year of age and allergic rhinitis (AR) at 4 years of age. A-C, DEP affects aeroallergen sensitivity at earlier ages, which is successively associated with AR at 4 years of age. DEP appears to be protective of AR at 4 year of age (red line). However, DEP is actually increasing the likelihood of aeroallergen sensitivity at 2 and 3 years of age, which then increases the risk of AR (black lines). A, The multivariable model at 1 year of age included ethnicity, breastfeeding duration, and Fel d 1 (low, medium, and high). B, The multivariable model at 2 years of age included ethnicity, breastfeeding duration, and children in the home at 1 year of age. C, The multivariable model at 3 years of age included breastfeeding duration at 3 years of age. Breastfeeding duration and breastfeeding duration × ethnicity interaction at 1 year of age were further evaluated to assess previous findings at 3 years of age.

Table 3
Profile Likelihood aORs (95% CIs) of allergen wheal area regression models at 2 and 3 years of age associated with allergic rhinitis at 4 years of age[a]

Aeroallergen	aORs (95% CIs) [*P* value]		
	Age of 2 years	Age of 3 years	Combined ages of 2 and 3 years
Timothy	1.06 (1.02–1.11) [.01]	…	…
Alternaria	1.07 (1.00–1.15) [.04]	…	1.11 (1.03–1.21) [.01]
Maple	…	1.04 (1.00–1.09) [.08]	1.05 (1.00–1.10) [.047]
Fescue	…	1.08 (1.02–1.15) [.02]	1.07 (1.01–1.15) [.03]
Dog	…	1.06 (1.01–1.12) [.03]	1.05 (1.00–1.11) [.08]
Penicillium	…	1.14 (1.06–1.23) [.001]	1.13 (1.05–1.22) [.002]

Abbreviations: CI, confidence interval; OR, odds ratio.
[a]Breastfeeding duration (months), Fel d 1 level in settled house dust, and season of birth covariables were included in the model for 2 years of age. In the model for 3 years of age and the model for the combined ages of 2 and 3 years, breastfeeding duration (months), Fel d 1 level in settled house dust, number of children in the home at 1 year of age, and ethnicity covariables were included.

Comparison of Aeroallergen-Specific Wheal Area Models to Summed Aeroallergen Wheal Area Models

After finding that specific allergen wheal areas were associated with AR, we asked whether the summed wheal area of all aeroallergens better fit the data than allergen-specific wheal areas. In the final multivariable model at 2 years of age, the sum of all 15 aeroallergen wheal areas was substituted for the timothy and *Alternaria* wheal areas. The allergen-specific wheal area model was significantly superior to the sum of all wheal areas of 15 aeroallergens (difference in log likelihood ratio of −36.8). Similarly at 3 years of age, the sum of all 15 aeroallergen wheal areas was substituted for maple, fescue, dog, and *Penicillium*. Again, the allergen-specific wheal area model was significantly superior to the total of all aeroallergen wheal areas (difference in log likelihood ratio of −50.7).

Sum of Allergen Wheal Areas in Final Regression Model (Informative Allergen Wheal Areas)

To further evaluate these findings, the sums of informative aeroallergen wheal areas at each age were examined (Fig 4). At 2 and 3 years of age, the sum of informative allergen wheal areas had a significant linear association with AR at 4 years of age (OR, 1.06; 95% CI, 1.03–1.10; $P < .001$; and OR, 1.07; 95% CI, 1.02–1.14; $P = .02$; respectively). At 2 years of age, we compared the interquartile range group and the high 75th percentile group with SPT-negative children, and a significant association was found with AR (OR, 2.12; 95% CI, 1.07–4.07; $P = .001$; and OR, 5.66; 95% CI, 2.12–15.92; $P < .001$; respectively) (Fig 4). Similarly, at 3 years of age, the 25th percentile, interquartile range, and 75th percentile children had elevated risks of AR compared with SPT-negative children (OR, 5.10; 95% CI, 2.47–10.48; $P < .001$; OR, 4.78; 95% CI, 2.65–8.60; $P < .001$; and OR, 8.87; 95% CI, 4.04–20.26; $P < .001$; respectively).

Discussion

This study of high-risk CCAAPS children found that AR prevalence increased annually to 21.9% by 4 years of age. To our knowledge, this is the first investigation of AR of high-risk children at 4 years of age. This percentage is higher than previously reported in population cohorts. The Isle of Wight general population–based study found the prevalence of rhinitis at 4 years of age to be 17.9%. Another UK population–based study at 5 years of age reported a prevalence of 12.1% for current rhinoconjunctivitis, a phenotype suggestive of AR.[32,33] Unlike our study, neither of the latter studies included SPT positivity as part of the AR case definition. Our AR case

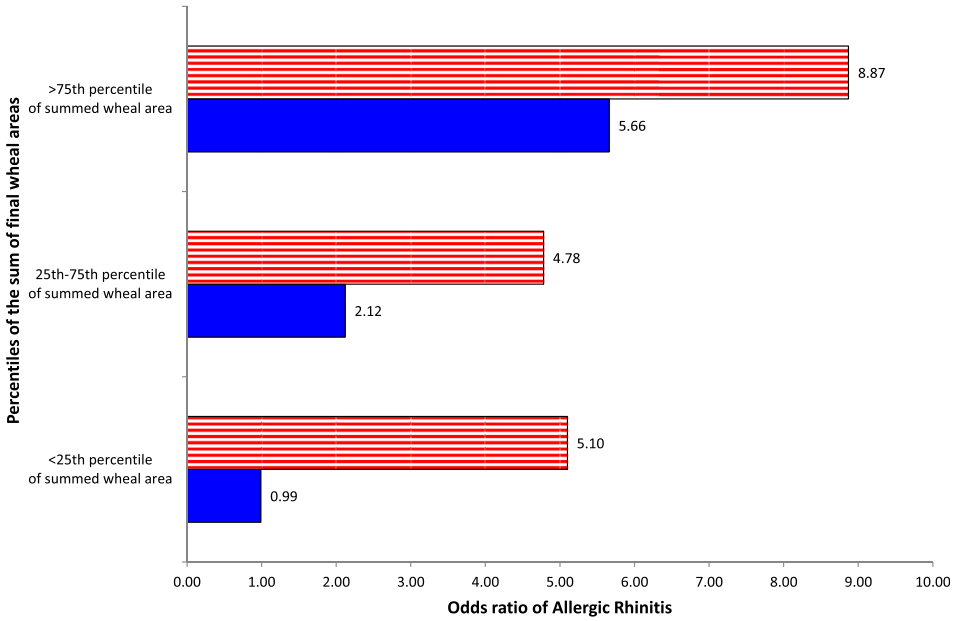

Figure 4. Odds ratios of developing allergic rhinitis at 4 years of age by percentiles of sum of informative allergen wheal areas from multivariable regression model at 1, 2, and 3 years of age. Allergen wheal areas that were included in the final multivariable model for the corresponding year were as follows: year 2: timothy and *Alternaria* for year 2 and maple, fescue, dog, and *Penicillium* for year 3. Blue bar indicates year 2; red striped bar, year 3.

definition better reflects the operational clinical criteria used by physicians to diagnose symptomatic children attending allergy clinics.

Aeroallergen sensitization at 2 years of age is a risk factor for AR, but not all sensitized children at 2 years of age have the same risk for AR at 4 years of age. Our results indicate that increases in the size of the wheal area of timothy grass allergen (*Phleum pratense*) and *Alternaria* at 2 years of age were significantly associated with AR. These results indicate that every 1-mm^2 increase in the wheal area of timothy and *Alternaria* increased the risk of AR by 6% and 7%, respectively. Regression models that used only timothy and *Alternaria* wheal areas were superior to models that totaled the wheal area of all aeroallergens. After summation of these wheal areas, a significant linear association persisted, and those with largest timothy-*Alternaria* wheal areas (ie, >75th percentile) had an almost 6-fold increased risk of AR compared with children who were timothy-*Alternaria* negative. At 3 years of age for every 1-mm^2 increase in the wheal area of maple, fescue grass, dog, and *Penicillium*, there was a 4%, 6%, 8%, and 14% increase in the risk of AR at 4 years of age, respectively. Similar to 2 years of age, a significant linear association was observed when the wheal areas of these 4 allergens at 3 years of age were summed to AR at 4 years of age. Also similar to 2 years of age, regression models at 3 years of age containing only maple, fescue, dog, and *Penicillium* wheal areas were superior to models that used the summed wheal area of all aeroallergens tested. Compared with SPT-negative children, the smallest (25th percentile) summed wheal area, the interquartile range of the summed area, and the largest summed wheal area all conferred significantly increased risk of AR at 4 years of age. This finding suggests that the magnitude of wheal reactions of select aeroallergens at specific ages best models the odds of developing AR. The stratified analysis suggests we are not detecting only early AR but also increased risk among atopic, asymptomatic children.

Allergen skin tests or in vitro specific IgE tests are essential for clinical evaluation of AR and for defining the AR phenotype in epidemiologic studies. A previous study found that case definitions of hay fever and current rhinitis based on questionnaires cannot be accurately measured or determined.[34] Recently, house dust mite sensitization at 1 and 2 years of age was associated with wheezing in 12-year-old children.[35] The observed ORs were large and had wide CIs, likely the result of dichotomizing the SPT result rather than measuring the wheal area. In our study, the continuous wheal area rather than the dichotomous response provided greater statistical power.[36,37] Studies using binary SPT results may imprecisely estimate the effect of the SPT.

We found that DEP exposure at 1 year of age was positively associated with aeroallergen sensitization at 2 and 3 years of age. At first glance, DEP exposure was inversely associated with AR, suggesting a protective effect on AR (upward diagonal red arrow in Figure 3A–C); however, this finding contradicts the postulated adjuvant effects of DEP.[38,39] After adjusting for other covariates, DEP at age 1 showed an bordeline association with aeroallergen sensitization at ages 2 and 3, though this was not statistically significant ($P = .07$). Aeroallergen sensitization was then associated with AR (Fig 3B and C). Directed acyclic graphs identify pathways to explain the association between independent and dependent variables.[40] We are the first to use and take advantage of directed acyclic graphs ability to identify pathways to explain the association between DEP exposure and aeroallergen sensitization and AR. Although we detected a trend that DEP exposure enhanced the likelihood of aeroallergen sensitization at 2 years of age ($P = .07$) and 3 years of age ($P = .07$), a retrospective power calculation found that we were underpowered (only powered at 46% at 2 years of age and 57% at 3 years of age) to detect an association. Our finding provides epidemiologic support for work by Diaz-Sanchez et al,[38] suggesting that DEP exposure may enhance sensitization to aeroallergens. Future studies investigating the association between DEP exposure and other allergic disorders (eg, allergic asthma and allergic eczema) with a positive SPT result as a component of the definitions should account for aeroallergen sensitization as an intermediate phenotype.

The large sample size and use of aeroallergen wheal areas are strengths of this study. Wheal area allows calculation of the mean diameter, which could be used in clinical practice beginning as early as 2 years of age. The reduced error with the wheal area compared with binary SPT values may improve model precision. If confirmed, this method could allow for more accurate risk prediction at each year for future AR. Poor parental recall limited our ability to study seasonal AR symptoms. However, our AR definition has been used in other similar birth cohorts.[2] Another potential limitation was that parental sensitization was not analyzed, but previous studies and unpublished data from our group found no association with child sensitization.[14] Our DEP was an estimate, and there may be error associated with our measurement; however, our group and other groups have used land-use regression in estimating fine-particulate matter exposure.[41,42] Because CCAAPS is a high-risk cohort, our findings may not be applicable to the general population.

In summary, in children of symptomatic and aeroallergen sensitized parents, the size of specific aeroallergen wheal areas at 2 and 3 years of age was associated with AR at 4 years of age. Regression models using specific aeroallergen wheal areas were superior to models summing the wheal areas of all aeroallergens. The risk of AR was greatest among those children with the largest sum of informative specific aeroallergens (timothy and *Alternaria* at 2 years of age and maple, dog, fescue, and *Penicillium* at 3 years of age). These novel findings in this high-risk group could improve identification of early childhood AR. Given that the association of AR with asthma and in childhood is mostly allergic, there is also the potential to improve asthma risk stratification.[43]

Supplementary Data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.anai.2014.10.020.

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eMethods

Continuous Independent Covariates

Logistic regression is similar to linear regression in assuming linearity of the independent predictor variables, which may not be true of continuous type variables. Smooth plots provide a graphical description of the relation between the continuous independent predictor and the dependent outcome. If there is no change in the relationship, the continuous independent predictor is included in the analysis without any modification. If the relationship between the independent predictor and the outcome changes over the range of values of the independent predictor (examples include bell-shaped or any curvilinear lines), then the independent predictor is divided into separate, continuous variables. The division will occur where the slope of the line changes direction, at the peaks and nadirs. These new variables will then have a linear relation with the dependent predictor over their subset range, while maintaining the power of being a continuous independent predictor. Inclusion of all the variables allows for the entire range of values of the independent predictor to be analyzed and will improve the overall fit of the model. This type of preparatory analysis was performed for the continuous independent covariates, which included endotoxin, β-glucan, Fel d 1, Can f 1, Der p 1, and Bla g 1). The exception to this was DEP, which was dichotomized at the 66th percentile, to be consistent with other analyses from the Cincinnati Childhood Allergy and Air Pollution Study group. For further information on logistic regression analysis, the readers are referred to other resources.[1,2]

Categorical Independent Covariates

Categorical independent predictor variables do not require a similar graphical analysis. These covariates were analyzed using logistic regression. If an association between the independent covariate and allergic rhinitis was found, at $P < .20$, the independent predictor was included in further multivariate analysis. If an association between the independent covariate and AR was found at $P < .20$, then the independent covariate was included in the multivariate analysis.

Skin Prick Test Allergen Wheal Area

As noted in the Methods section, logistic regression was used to analyze all 15 aeroallergens and the 2 food allergens. If an association between the independent allergen wheal area and AR was found, at $P < .20$ after adjustment for multiple comparisons using the Holms-Sidak test, the independent allergen wheal area was included in further multivariate analysis.

Multivariate Analysis

Multivariate logistic regression was performed for covariates and each age of SPT wheal area, such as 1, 2, or 3 years of age, for testing of associations with allergic rhinitis at 4 years of age. The "all-subsets" method was used to reduce the number of variables to those that were informative and allowed for best fit.[3] The criteria for removing a variable was if the log likelihood ratio of the reduced model did not decrease significantly and/or the remaining variable coefficients did not change by more than 20%.

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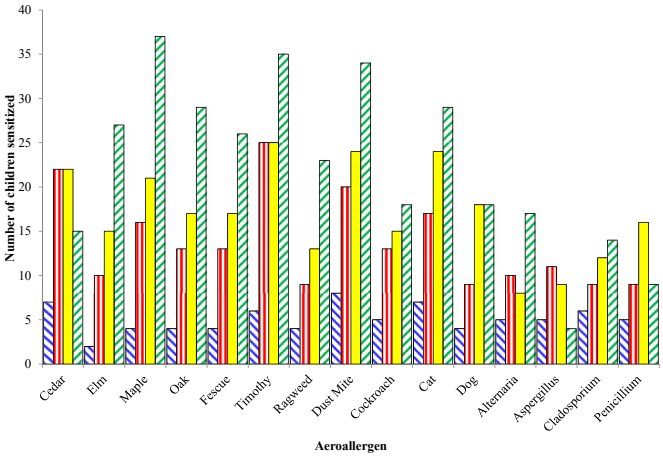

eFigure 1. Absolute number of aeroallergen sensitivities among allergic rhinitis children at ages one to four.

eTable 1
P values for the holm-sidak test–adjusted skin prick test wheal area at 1, 2, and 3 years of age associated with allergic rhinitis (compared with all other phenotypes) at 4 years of age[a]

Allergen	Age of 1 year (n = 596)	Age of 2 years (n = 591)	Age of 3 years (n = 609)
Pollens			
Cedar	.84	.65	.48
Elm	>.99	.88	.01[b]
Maple	>.99	.18[c]	.02[b]
Oak	>.99	.62	.40
Fescue	>.99	.97	.01[b]
Timothy	>.99	.09[c]	.08[b]
Ragweed	.94	.76	.86
Arthropods			
Dust mite	>.99	.92	.70
Cockroach	>.99	>.99	.98
Mammals			
Cat	.61	.16[c]	.43
Dog	>.99	.99	.17[b]
Molds			
Alternaria	>.99	.01[b]	.50
Aspergillus	>.99	.73	.79
Cladosporium	.96	>.99	>.99
Penicillium	>.99	.90	.01[b]
Foods			
Milk	>.99	>.99	>.99
Egg	>.99	.85	.99

[a]In each year there was at least 1 child whose parent refused testing to milk and egg. Therefore, the denominator for the 1 year of age group is 595; for the age of 2 years, the denominator is 589 (for milk) and 588 (for egg); and for the age of 3 years group, the denominator is 609 (for milk) and 602 (for egg).
[b]$P < .05$.
[c]$P < .20$.

eTable 2
P values for the unadjusted association of allergen wheal areas at 1, 2, and 3 years of age (stratified to those who were aeroallergen sensitized but asymptomatic) with allergic rhinitis at 4 years of age

Allergen	Age of 1 year (n = 67)	Age of 2 years (n = 117)	Age of 3 years (n = 141)
Pollens			
Cedar	.99	.40	>.99
Elm	>.99	.94	.10
Maple	>.99	>.99	>.99
Oak	>.99	>.99	>.99
Fescue	>.99	>.99	>.99
Timothy	>.99	.98	>.99
Ragweed	…	>.99	>.99
Arthropods			
Dust mite	>.99	>.99	>.99
Cockroach	…	>.99	>.99
Mammals			
Cat	.97	>.99	>.99
Dog	…	>.99	>.99
Molds			
Alternaria	>.99	>.99	>.99
Aspergillus	…	>.99	.99
Cladosporium	>.99	…	>.99
Penicillium	>.99	>.99	>.99
Foods			
Milk	>.99	…	…
Egg	>.99	>.99	>.99

eTable 3
P values for the unadjusted association of allergen wheal areas at 1, 2, and 3 years of age (stratified to those with early allergic rhinitis) with allergic rhinitis at 4 years of age

Allergen	Age of 1 year (n = 41)	Age of 2 years (n = 100)	Age of 3 years (n = 109)
Pollens			
Cedar	>.99	.79	>.99
Elm	…	>.99	>.99
Maple	>.99	.71	.23
Oak	>.99	>.99	>.99
Fescue	>.99	>.99	.79
Timothy	.95	>.99	.98
Ragweed	>.99	>.99	>.99
Arthropods			
Dust mite	…	>.99	>.99
Cockroach	>.99	>.99	>.99
Mammals			
Cat	>.99	.97	>.99
Dog	>.99	.95	.93
Molds			
Alternaria	>.99	.002[a]	>.99
Aspergillus	.65	.97	>.99
Cladosporium	.87	>.99	>.99
Penicillium	>.99	>.99	>.99
Foods			
Milk	>.99	>.99	>.99
Egg	>.99	.78	>.99

[a]$P < .003$.

eTable 4
Stratified aORs of allergen wheal area at 2 and 3 years of Age[a]

Variable	Allergen	aORs (95% CIs) [*P* value]	
		Age of 2 years	Age of 3 years
Sensitized but not symptomatic strata	Elm	…	1.08 (1.03–1.21) [.01]
Sensitized and symptomatic (allergic rhinitis) strata	*Alternaria*	1.19 (1.05–1.48) [.03]	…

Abbreviations: aOR, adjusted odds ratio; CI, confidence interval.
[a]Stratified analyses were performed for aeroallergen sensitivity associated with allergic rhinitis at 4 years of age. First analysis was among children who were sensitized but not symptomatic. At 2 years of age, no aeroallergen wheal area was significant in multivariable model. At 3 years of age, elm wheal area size was significantly associated with AR at 4 years of age, with covariates including β-glucan, duration of breastfeeding, and season of birth. The second analysis was among children who were sensitized and symptomatic (ie, had early onset of allergic rhinitis). At 2 years of age, *Alternaria* wheal area was significantly associated with allergic rhinitis at 4 years of age, with covariates including ethnicity, duration of breastfeeding, and number of children in home at 1 year of age. At 3 years of age, no aeroallergen was significant in the multivariable model.