

## Breast-feeding, aeroallergen sensitization, and environmental exposures during infancy are determinants of childhood allergic rhinitis

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**Background:** Infant predictors of early childhood allergic rhinitis (AR) are poorly understood.

**Objective:** We sought to identify environmental exposures and host factors during infancy that predict AR at age 3 years.

**Methods:** High-risk children from greater Cincinnati were followed annually from ages 1 to 3 years. AR was defined as sneezing, runny, or blocked nose in the prior 12 months and a positive skin prick test (SPT) response to 1 or more aeroallergens. Environmental and standardized medical questionnaires determined exposures and clinical outcomes. Primary activity area dust samples were analyzed for house dust endotoxin (HDE) and (1-3)- $\beta$ -D-glucan. Fine particulate matter sampled at 27 monitoring stations was used to estimate personal elemental carbon attributable to traffic exposure by using a land-use regression model.

**Results:** Of 361 children in this analysis, 116 had AR, and 245 were nonatopic and nonsymptomatic. Prolonged breast-feeding

in African American children (adjusted odds ratio [aOR], 0.8; 95% CI, 0.6-0.9) and multiple children in the home during infancy was protective against AR (aOR, 0.4; 95% CI, 0.2-0.8). Food SPT response positivity and tree SPT response positivity in infancy increased the risk of AR at age 3 years (aOR of 4.4 [95% CI, 2.1-9.2] and aOR of 6.8 [95% CI, 2.5-18.7], respectively). HDE exposure was associated with AR; the effect was dependent on exposure level. Elemental carbon attributable to traffic and environmental tobacco smoke exposure showed no effect on AR.

**Conclusion:** Prolonged breast-feeding in African American subjects and multiple children in the home during infancy reduced the risk of AR at age 3 years. SPT response positivity to food and tree allergens enhanced risk. The HDE effect on AR was related to exposure. (J Allergy Clin Immunol 2010;125:1054-60.)

**Key words:** Tobacco, atopy, rhinitis, endotoxin, breast-feeding, allergy, diesel, siblings, infants, African American

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Upward of 40% of children are afflicted with allergic rhinitis (AR), with direct costs estimated at \$11 billion in 2005.<sup>1,2</sup> Children with AR symptoms are at risk for asthma.<sup>3</sup> The environmental factors associated with the development of AR are not well defined. Adolescents living near high truck traffic experience a higher frequency of AR symptoms.<sup>4</sup> Human experimental studies demonstrated that diesel exhaust particles can induce or enhance nasal specific IgE and T<sub>H</sub>2 cytokine responses to allergens.<sup>5-7</sup> However, no prospective studies have investigated the combined effects of outdoor pollutants, including diesel exhaust particles, and multiple indoor exposure variables on childhood AR.

The Cincinnati Childhood Allergy and Air Pollution Study (CCAAPS) is a prospective high-risk birth cohort study of children born to atopic parents designed to determine the effects of infant and early childhood environmental exposures, including diesel pollution, on allergic disorders.<sup>8</sup> The present study's objective is to determine the interrelationship of host factors and outdoor and indoor environmental exposures in early life on the development of AR at age 3 years.

## METHODS

### Subject recruitment and study design

The CCAAPS cohort has been previously detailed.<sup>9</sup> Infants born in the greater Cincinnati and northern Kentucky area were identified from birth records and recruited between 2001 and 2003. Parents living either within 400 m

#### Abbreviations used

aOR: Adjusted odds ratio

AR: Allergic rhinitis

CCAAPS: Cincinnati Childhood Allergy and Air Pollution Study

ECAT: Elemental carbon attributable to traffic

ETS: Environmental tobacco smoke

EU: Endotoxin units

HDE: House dust endotoxin

OR: Odds ratio

PM<sub>2.5</sub>: Particulate matter with diameter equal to or less than 2.5  $\mu$ m

SPT: Skin prick test

or greater than 1,500 m of a major road were invited to a screening visit at one of 3 clinical study sites. At that visit, parents provided informed consent and then were administered an allergy symptom questionnaire and received skin prick testing to a panel of aeroallergens. If 1 or both parents reported rhinitis, eczema, or asthma and exhibited a positive skin prick test (SPT) response to at least 1 regional aeroallergen, their infant was included in the study. The study protocol and informed consent statement were approved by the University of Cincinnati Institutional Review Board.

Parents and infants returned to the clinical study site at age 1 year (mean age, 13.7  $\pm$  2.6 months) for their first annual comprehensive study visit. Parents completed a medical history and home environmental questionnaire pertaining to their infants. The infant received a physical examination and underwent skin prick testing to a panel of 15 aeroallergens, including white oak, elm, maple mix, eastern red cedar, fescue and timothy grasses, short ragweed, 4 mold allergens (*Alternaria alternata*, *Aspergillus fumigatus*, *Penicillium* species mix, and *Cladosporium* species), cat, dog, house dust mite mix (*Dermatophagoides farinae* and *Dermatophagoides pteronyssinus*), and German cockroach (ALK-Abelló, Round Rock, Tex). Two food allergens, milk and egg white, were also tested. A wheal diameter at least 3 mm greater than that elicited by the negative saline control was considered positive.

At the follow-up comprehensive clinic visit at age 3 years, children were re-evaluated (by means of questionnaires administered to parents), examined, and skin tested. Child respiratory symptoms were captured by using items adapted from the validated International Study of Asthma and Allergies in Childhood questionnaire.<sup>10</sup> The primary study outcome of AR at age 3 years was defined as a positive parental response to the 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?" and a positive SPT response to 1 or more aeroallergens. Subjects with AR were compared with children without symptoms and negative SPT responses to all 15 aeroallergens at age 3 years. The secondary outcome of atopy at age 3 years was defined as a positive SPT response to at least 1 aeroallergen and compared with children with negative SPT responses to all aeroallergens. The secondary outcome of rhinitis at age 3 years was defined by parental report of symptoms to the aforementioned rhinitis questionnaire item, and the comparator group included children without symptoms.

## Home evaluations

When the infants were 8 months old, their homes were inspected, and house dust samples were collected from the primary living area.<sup>11</sup> A 2-m<sup>2</sup> area of floor surface in the infant's primary activity room was vacuumed with a Filter Queen Majestic vacuum cleaner (Health-Mor; HMI Industries, Inc, Seven Hills, Ohio) at a rate of 2 min/m<sup>2</sup>. Samples were filtered through a 355- $\mu$ m pyrogen-free screen, desiccated, and stored at  $-20^{\circ}\text{C}$ . Filtered house dust endotoxin (HDE) and (1-3)- $\beta$ -D-glucan concentrations were determined by using the limulus amoebocyte lysate assay (Associates of Cape Cod, Inc, Falmouth, Mass).<sup>11,12</sup> HDE was expressed as endotoxin units (EU) per milligram of dust, whereas (1-3)- $\beta$ -D-glucan was expressed as micrograms per gram of dust. Filtered house dust was tested for Fel d 1 (microgram per gram of dust) by using a monoclonal sandwich ELISA assay (Indoor Biotechnologies, Inc,

Charlottesville, Va).<sup>13</sup> Indoor exposure variables were treated as continuous predictors.

## Outdoor exposure estimation

Outdoor tree and grass pollens, ragweed pollen, and fungal spores were sampled with a Rotorod Sampler (Sampling Technologies, Inc, Minnetonka, Minn). The method of quantification and identification of pollens and fungal spores was previously reported.<sup>14,15</sup> Outdoor exposure variables were treated as continuous predictors.

## Traffic exposure estimation

Ambient air sampling was performed from 2001 through 2005 at 27 sites in southwest Ohio and northern Kentucky.<sup>16</sup> Particulate matter of 2.5  $\mu$ m in diameter or less (PM<sub>2.5</sub>) was collected on 37-mm membrane Teflon filters (nominal pore size, 1  $\mu$ m; Paul Gellman, Ann Arbor, Mich) and 37-mm Quartz filters with Harvard-type Impactors (Air Diagnostics and Engineering, Harrison, Me). Quartz filters, Teflon filters, or both were used to determine PM<sub>2.5</sub> elemental composition.<sup>17</sup> Teflon filters were also used to determine particulate mass and the elemental carbon component of PM<sub>2.5</sub>. A marker of traffic-related air pollution, elemental carbon attributable to traffic (ECAT), was determined at each sampling location by using the multivariate UNMIX model and chemical mass balance model.<sup>17</sup> Using the surrounding land-use and traffic characteristics, a land-use regression model was developed to predict average daily ECAT levels (in micrograms per cubic meter) at each of the 27 monitoring stations.<sup>18</sup> The land-use regression model was then applied to each child's residence and other locations where the child spent more than 8 hours per week to derive a time-weighted average daily exposure to ECAT.<sup>18</sup> ECAT exposure was treated as a continuous variable.

## Predictor variables

The CCAAPS cohort consists of 16% African American children, 81% white children, and 3% "other" (eg, biracial and Pacific Islander) children. We *a priori* grouped white children with the 3% "other" children in the non-African American category. Risk factors and exposures evaluated included the following: race (African American and non-African American); sex; household income at age 1 year (<\$20,000 and  $\geq$ \$20,000); breast-feeding duration (number of months); number of other children living in the home at age 1 year (<2 and  $\geq$ 2); report of parental rhinitis symptoms at initial visit (no and yes); child's season of birth; presence of 1 or more dogs in the home at age 1 year (no and yes); environmental tobacco smoke (ETS) estimated from parental report of the number of cigarettes smoked by all household members at age 1 year (0, 1-19, and  $\geq$ 20); ECAT exposure; airborne pollen and fungal spores; SPT response positivity to trees at age 1 year; and SPT response positivity to milk, egg, or both at age 1 year. Exposure to ETS was analyzed as a categorical variable because of potential recall and reporting bias.

## Data analysis

Categories of predictor variables were evaluated for association with AR by using the Pearson  $\chi^2$  test (Table 1) or the Fisher exact test (see Table E1 in this article's Online Repository at [www.jacionline.org](http://www.jacionline.org)). Categorical and continuous predictor variables were evaluated by means of univariate logistic regression. Collinearity was assessed by using correlation coefficients. The scale for modeling each continuous predictor variable was determined by fitting a smooth function to the data. Linear splines were modeled on separate intervals at which the slopes changed, as determined by significance testing and visual assessment with S-Plus 2000 software (TIBCO Software, Inc, Palo Alto, Calif).<sup>19</sup> A final logistic regression model was developed by using predictor variables identified with AR by means of univariate analysis. A *P* value of less than .15 was chosen for inclusion in a multiple logistic regression model. Variables maintained in the final multiple regression model were chosen by using the "all subsets" method of stepwise regression and removed if the *P* value was greater than 0.1, except if (1) the model improved by log likelihood ratio and (1) likelihood coefficients of remaining variables changed by 20%.

**TABLE I.** Subjects' characteristics and risk factors for AR, atopy, and rhinitis at age 3 years

Variable in infancy	AR* (n = 116/361 [32%])		Atopy† (n = 267/606 [44%])		Rhinitis‡ (n = 210/606 [35%])	
	No. (%)	P value	No. (%)	P value	No. (%)	P value
Sex						
Female	52 (31)		117 (42)		95 (34)	
Male	64 (34)	.6	150 (46)	.4	115 (35)	.8
Race						
Non-African American	83 (30)		200 (42)		161 (34)	
African American	33 (41)	.05	67 (52)	.05	49 (38)	.4
Season of birth						
Winter	21 (22)		77 (42)		51 (28)	
Spring	33 (40)	.01	58 (45)	.6	55 (43)	.01
Summer	24 (36)	.05	43 (43)	1.0	39 (39)	.07
Autumn	38 (33)	.08	89 (46)	.5	65 (34)	.3
Family income						
≥\$20,000	84 (29)		199 (42)		160 (33)	
<\$20,000	18 (40)	.1	40 (49)	.2	32 (40)	.3
Children in home						
0-1	91 (36)		199 (47)		153 (36)	
≥2	25 (23)	.01	68 (37)	.02	57 (31)	.2
Day care						
No	67 (32)		160 (45)		117 (33)	
Yes	34 (30)	.7	79 (40)	.2	73 (37)	.4
SPT response to milk and/or egg						
Negative	88 (28)		214 (41)		173 (33)	
Positive	28 (58)	<.001	53 (65)	<.001	37 (45)	.03
SPT response to trees§						
Negative	100 (30)		244 (43)		190 (33)	
Positive	16 (67)	<.001	23 (66)	.01	20 (57)	<.01
ETS (cigarettes/d)						
0	75 (30)		194 (44)		141 (32)	
1-19	13 (38)		25 (42)		26 (44)	
≥20	13 (33)	.6	19 (35)	.4	23 (41)	.1
ECAT (μg/m <sup>3</sup> )						
<0.343	53 (29)		127 (41)		106 (34)	
≥0.343	63 (35)	.2	140 (47)	.2	104 (35)	.9
Dogs in home						
≥1	81 (34)		181 (46)		135 (35)	
0	35 (29)	.4	86 (41)	.2	75 (35)	.8

(Continued)

Logistic regression models using (1) the same original predictors in the primary AR outcome and (2) predictors irrespective of significance to AR were developed for the secondary outcomes of atopy and rhinitis. All analyses were performed with SAS 9.1.3 software (SAS Institute, Inc, Cary, NC).

## RESULTS

### Demographics and exposure characteristics

At age 3 years, 606 children completed medical evaluations and skin prick testing. The outcome definition of AR was found in 116 (19%) children, and 245 (40%) nonatopic asymptomatic children were included in the comparator group. No further primary outcomes analysis was conducted on the 151 (25%) atopic asymptomatic children and 94 (16%) nonatopic symptomatic children. The mean age of the AR group and the comparison group was  $36.9 \pm 1.7$  and  $36.7 \pm 1.6$  months, respectively. Among the 361 subjects in the AR and comparator groups, 80 (22.2%) infants were African American, and 281 (77.8%) were non-African American (278 of these were white). Of these 361 children, there were 191 male (52.9%) and 170 female (47.1%) children. Of the AR case and comparator children about whom

there was information regarding those who were breast-fed and when solids were introduced, 95 (29.5%) infants were exclusively breast-fed, whereas 227 (70.5%) were breast-fed with solids. Of all 606 three-year-old children, 130 (21.5%) were African American, and 476 (78.6%) were non-African American. Table E1 shows that regardless of whether 1 or both parents were skin tested or whether 1 or both parents tested had positive skin test responses, the frequencies of AR, atopy, and rhinitis outcomes did not significantly differ.

### Univariate evaluation of predictors of primary clinical outcome: AR at age 3 years

Subject characteristics are presented in Table I. Characteristics significantly associated with AR and evaluated further in multivariate analysis included season of birth (being born during the spring [odds ratio [OR], 2.4; 95% CI, 1.2-4.6] or summer [OR, 2.0; 95% CI, 0.99-4.0]); 2 or more children in the home during infancy (OR, 0.5; 95% CI, 0.3-0.9); a positive SPT response to milk, egg, or both in infancy (OR, 3.6; 95% CI, 1.9-6.7); and a positive SPT response to any tree pollen in infancy (OR, 4.7; 95% CI, 2.0-11.4). Breast-feeding duration was protective in

TABLE I. (Continued)

Variable in infancy	AR* (n = 116/361 [32%])		Atopy† (n = 267/606 [44%])		Rhinitis‡ (n = 210/606 [35%])	
	No. (%)	P value	No. (%)	P value	No. (%)	P value
Fel d 1 (μg/g dust)						
<0.69749	62 (31)		140 (43)		110 (34)	
≥0.69749	54 (34)	.6	127 (45)	.6	100 (36)	.6
Mold (spores/m <sup>3</sup> )						
<490,408	52 (29)		135 (44)		98 (32)	
≥490,408	64 (35)	.2	132 (44)	1.0	112 (37)	.2
Tree and grass pollen (pollen/m <sup>3</sup> )						
<37,583	56 (32)		138 (45)		108 (35)	
≥37,583	60 (32)	1.0	129 (43)	.8	102 (34)	.9
Ragweed pollen (pollen/m <sup>3</sup> )						
<3,243	116 (32)		267 (44)		210 (35)	
≥3,243	0	NA	0	NA	0	NA
(1-3)-β-D-glucan (μg/g dust)						
<55.5	76 (36)		166 (47)		130 (37)	
≥55.5	40 (26)	.04	101 (40)	.08	80 (32)	.2
Breast-feeding duration (mo)						
<4	71 (36)		148 (45)		122 (37)	
≥4	45 (28)	.1	119 (43)	.6	88 (32)	.2
HDE (EU/mg dust)						
6-36.6	47 (35)		95 (45)		76 (36)	
36.6-244.7¶	58 (31)		150 (45)		114 (34)	
≥244.7#	11 (27)	.6	22 (36)	.4	20 (33)	.9

Note: P values were obtained by using the Pearson  $\chi^2$  test of independence.

NA, Not able to be tested because of zero count.

\*AR = "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?" AND positive SPT response to 1 or more of the 15 aeroallergens tested.

†Atopy = positive SPT response to 1 or more of the 15 aeroallergens tested.

‡Rhinitis = "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?"

§Trees tested included white oak (*Quercus alba*), American elm (*Ulmus Americana*), maple mix, and Eastern red cedar (*Juniperus virginiana*).

||Low endotoxin level ranged from 6 to 36.6 EU/mg dust.

¶Medium endotoxin level ranged from 36.6 to 244.7 EU/mg dust.

#High endotoxin level was greater than 244.7 EU/mg dust.

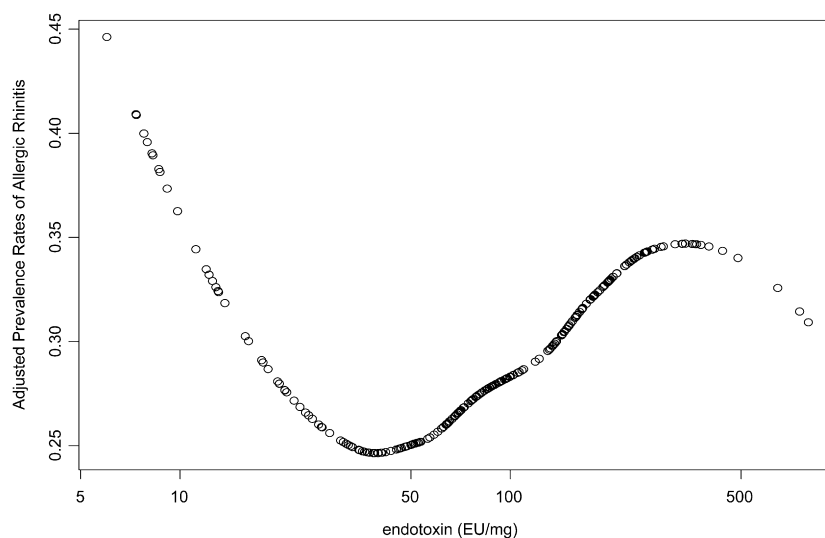


FIG 1. Smooth plot of AR prevalence in relation to HDE concentration.

African American subjects (OR, 0.8; 95% CI, 0.7-0.97). Low annual family income (<\$20,000) was significantly correlated with having at least 1 African American biological parent ( $r = 0.5$ ,  $P < .0001$ ) and therefore not included.

Smoothed plots were used to determine the univariate associations of environmental exposures with AR. HDE concentrations ranged from 6 EU/mg (the limit of detection) to 800 EU/mg, with a geometric mean of 212 EU/mg dust. HDE

**TABLE II.** aORs for predictors of AR, atopy, and rhinitis at age 3 years

Variable at age 1 y	AR* (n = 100/322 [31%]), aOR (95% CI)	Atopy† (n = 238/549 [43%]), aOR (95% CI)	Rhinitis‡ (n = 189/549 [34%]), aOR (95% CI)
Breast-feeding duration (mo) in African American subjects	0.8   (0.6-0.9)	0.9 (0.9-1.0)	0.8§ (0.7-0.9)
Breast-feeding duration (mo) in non-African American subjects	1.0 (0.96-1.1)	1.01 (0.97-1.1)	1.0 (0.96-1.1)
Season of birth			
Winter	1.0	1.0	1.0
Autumn	2.2§ (1.0-4.7)	1.2 (0.8-1.9)	1.4 (0.9-2.2)
Spring	2.9§ (1.3-6.6)	1.1 (0.6-1.8)	2.0§ (1.2-3.4)
Summer	2.1 (0.9-4.9)	0.8 (0.5-1.4)	1.7 (1.0-3.0)
Positive SPT response to trees in infancy	8.7¶ (3.0-24.8)	3.3   (1.5-7.4)	2.6§ (1.2-5.4)
Positive SPT response to milk and/or egg in infancy	4.5¶ (2.1-9.8)	2.8¶ (1.7-4.7)	1.6 (0.98-2.7)
≥2 Children in home in infancy	0.5§ (0.3-0.9)	0.6§ (0.4-0.9)	0.8 (0.5-1.2)
Low HDE (EU/mg)# in infancy	0.5§ (0.3-0.8)	0.8 (0.5-1.1)	0.8 (0.5-1.1)
Medium HDE (EU/mg)** in infancy	6.3¶ (2.3-17.2)	2.1§ (1.1-4.0)	1.8 (1.0-3.5)
High HDE (EU/mg)†† in infancy	0.002   (<0.001- 0.1)	0.1   (0.03-0.5)	0.4 (0.1-1.5)

Covariates included race; breast-feeding duration; race\*breast-feeding duration; season of birth; tree SPT response positivity; milk, egg, or both SPT response positivity; number of children in the home during infancy; low endotoxin levels in infancy; and high endotoxin levels in infancy. The main effects of race and breast-feeding duration were included in the model. The ORs of the main effects of race and breast-feeding variables were determined by means of modeling without an interaction term, but these were not significant. \*AR = "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?" AND positive SPT response to 1 or more of the 15 aeroallergens tested.

†Atopy = positive SPT response to 1 or more of the 15 aeroallergens tested.

‡Rhinitis = "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?"

§ $P < .05$ .

|| $P < .01$ .

¶ $P < .001$ .

#Low endotoxin level ranged from 6 to 36.6 EU/mg dust.

\*\*Medium endotoxin level ranged from 36.6 to 244.7 EU/mg dust.

††High endotoxin level was greater than 244.7 EU/mg dust.

concentrations were log transformed to approximate normal distribution. As shown in Fig 1, the HDE smooth plot was significantly associated with AR ( $\chi^2 = 6.4$ ,  $P = .01$ ). HDE concentrations were protective against AR at less than 36.6 EU/mg and greater than 244.7 EU/mg while associated with AR at between 36.6 and 244.7 EU/mg. Therefore HDE was included in the multivariate model as low HDE, medium HDE, or high HDE.

ECAT exposure was log transformed to approximate a normal distribution. A smooth plot of ECAT was associated with AR ( $\chi^2 = 3.7$ ,  $P = .05$ ), and suggested levels of less than  $0.31 \mu\text{g}/\text{m}^3$  were protective, whereas those greater than  $0.31 \mu\text{g}/\text{m}^3$  were prone to develop AR. No significant associations with AR were found for Fel d 1 cat allergen ( $\chi^2 = 0.3$ ,  $P = .6$ ), number of dogs in the home ( $\chi^2 = 2.0$ ,  $P = .16$ ), (1-3)- $\beta$ -D-glucan ( $\chi^2 = 1.2$ ,  $P = .3$ ), fungal spore counts ( $\chi^2 = 2.0$ ,  $P = .15$ ), ragweed pollen counts ( $\chi^2 = 1.2$ ,  $P = .2$ ), tree and grass pollen counts ( $\chi^2 = 1.2$ ,  $P = .3$ ), and ETS ( $\chi^2 = 0.8$ ,  $P = .4$ ).

### Multivariate evaluation of predictors of primary clinical outcome: AR at age 3 years

Multivariate regression results are shown in Table II. Covariates in the final multivariate regression model included: race; breast-feeding duration; race\*breast-feeding duration interaction; season of birth; tree SPT response positivity; milk, egg, or both SPT response positivity; 2 or more children in the home during infancy; low endotoxin level; and high endotoxin level. Prolonged breast-feeding in African American subjects was protective against AR (adjusted odds ratio [aOR], 0.8; 95% CI, 0.6-0.9). SPT response positivity to any tree pollen in infancy (aOR, 8.7;

95% CI, 3.0-24.8); SPT response positivity to milk allergen, egg allergen, or both in infancy (aOR, 4.5; 95% CI, 2.1-9.8); being born in the autumn months (aOR, 2.2; 95% CI, 1.1-4.9); and being born in the spring months (aOR, 2.9; 95% CI, 1.3-6.6) were each significantly associated with AR at age 3 years. Infants with 2 or more other children in the home were significantly less likely to have AR (aOR, 0.5; 95% CI, 0.3-0.9). Low HDE and high HDE exposure in infancy was protective against AR (aOR, 0.5; 95% CI, 0.3-0.8) and (aOR, 0.002; 95% CI, <0.001-0.1), respectively. Medium HDE exposure during infancy was associated with a significantly increased risk of AR (aOR, 6.3; 95% CI, 2.3-17.2).

### Multivariate evaluations of AR risk factors on secondary outcomes of atopy and rhinitis

We aimed to determine whether the effect of a predictor on AR was determined by the skin test positivity component (ie, atopy) or the symptom component (ie, rhinitis). Multivariate models for atopy and rhinitis were built using only those predictors found significant for AR. Of AR predictors, those found to be associated with atopy at age 3 years (Table II) included SPT response positivity to any tree pollen at age 1 year (aOR, 3.1; 95% CI, 1.4-6.9) and SPT response positivity to milk, egg, or both at age 1 year (aOR, 2.9; 95% CI, 1.7-4.8). Multiple children in the home significantly reduced the risk for atopy at age 3 years (aOR, 0.6; 95% CI, 0.4-0.9). Medium and high HDE concentrations were associated with atopy at age 3 years (aOR, 2.1 [95% CI, 1.1-4.0] and aOR, 0.1 [95% CI, 0.03-0.5], respectively). There was no association with low HDE exposure.

Of AR risk factors, those found to be associated with rhinitis at age 3 years (Table II) included spring season of birth (aOR, 2.0;



95% CI, 1.2-3.4), summer season of birth (aOR, 1.8; 95% CI, 1.0-3.1), and SPT response positivity to any tree pollen at age 1 year (aOR, 2.5; 95% CI, 1.2-5.2). Prolonged breast-feeding in African American infants significantly reduced the risk of rhinitis symptoms at age 3 years (aOR, 0.8; 95% CI, 0.7-0.9). Low, medium, or high HDE concentrations were not associated with rhinitis at age 3 years.

### Independent multivariate evaluations of secondary outcomes of atopy and rhinitis

Multivariate models of atopy and rhinitis were developed independent of AR. Significant predictors associated with atopy included SPT response positivity to any tree pollen in infancy (aOR, 3.3; 95% CI, 1.5-7.2); SPT response positivity to milk, egg, or both in infancy (aOR, 2.8; 95% CI, 1.7-4.7); multiple children in the home in infancy (aOR, 0.6; 95% CI, 0.4-0.9); medium HDE exposure (aOR, 2.1; 95% CI, 1.1-3.8); and high HDE exposure (aOR, 0.1; 95% CI, 0.03-0.5). Nonsignificant independent variables remaining in the atopy model include low HDE exposure, prolonged breast-feeding in African American infants, and prolonged breast-feeding in non-African American infants. Significant predictors found associated with rhinitis include spring season of birth (aOR, 2.6; 95% CI, 1.4-4.7), summer season of birth (aOR, 2.1; 95% CI, 1.1-4.0), SPT response positivity to any tree pollen at age 1 year (aOR, 2.4; 95% CI, 1.1-5.1), and prolonged breast-feeding in African American infants (aOR, 0.8; 95% CI, 0.7-0.96). Nonsignificant independent variables remaining in the rhinitis model included low HDE exposure; medium HDE exposure; high HDE exposure; SPT response positivity to milk, egg, or both in infancy; and prolonged breast-feeding in non-African American infants.

## DISCUSSION

In this CCAAPS birth cohort study of 3-year-old children, the prevalence of AR was 19%. This is greater than the 5% prevalence of seasonal AR at age 3 years previously reported in another high-risk birth cohort study.<sup>20</sup> Differences in prevalence estimates might be due to the use of timothy- and birch serum specific IgE to define sensitization (and seasonal AR) in the latter study, in contrast to our study that used skin prick testing to 15 aeroallergens.<sup>21</sup>

Another high-risk birth cohort study previously showed that African American subjects were at significantly greater risk than white subjects for physician-diagnosed AR at age 5 years.<sup>22</sup> We showed that African American infants receiving prolonged breast-feeding had significantly decreased risk of AR at age 3 years (aOR, 0.8; 95% CI, 0.6-0.9). This breast-feeding effect was not seen in non-African American children (aOR, 1.0; 95% CI, 0.96-1.1). The effect was independent of income because when we substituted income for race in the multivariate model, no significant association was observed.

This protective effect of prolonged breast-feeding in African American subjects has not been investigated or reported in other high-risk cohort studies. The Third National Health and Nutrition Examination Survey and the 2004 National Immunization Survey reported that breast-feeding rates are lower in African American infants and particularly in lower-socioeconomic-status subgroups.<sup>23,24</sup> The Breastfeeding Promotion Consortium, which includes the African-American Breastfeeding Alliance, promotes breast-feeding in women and African Americans.<sup>25-27</sup> If confirmed,

this finding could guide future recommendations for primary prevention of allergic disorders in African American children, which could be disseminated through the aforementioned advocacy groups.

Indoor HDE exposure in the first year of life was associated with AR at age 3 years. The bimodal effect of HDE in the univariate analysis persisted in the multivariate model. The risk of AR at age 3 years was decreased with low HDE exposure in infancy (6-36.6 EU/mg), but the risk increased with medium HDE exposure (36.6-244.7 EU/mg). The risk of AR at age 3 years was decreased with levels of 244.7 EU/mg or greater; however, only 11 children with AR were exposed to HDE concentrations in this range, and therefore these data should be interpreted with caution. Although we have previously shown that HDE concentrations correlate with the presence of multiple dogs, the effect of HDE concentrations on AR at age 3 years was independent of pet keeping; that is, the presence of multiple dogs was not significantly associated with AR nor did it modify the HDE effect on AR in this multivariate model (data not shown).<sup>11</sup> The atopy and rhinitis secondary outcomes, although not significant, both trended to an increased risk with higher HDE exposure, which agreed with the primary AR outcome. It can be hypothesized that endotoxin could play an adjuvant role, enhancing early respiratory sensitization to aeroallergens. Experimental murine models showing that LPS is essential for ovalbumin-induced respiratory sensitization lend support to this hypothesis.<sup>28,29</sup>

We found that infants with SPT positivity to milk, eggs, or both in infancy were 4 times more likely to have AR at age 3 years. Other childhood studies have indicated that egg sensitization in infancy is a predictor of allergic respiratory disorders later in life.<sup>30</sup> Rhodes et al<sup>31</sup> demonstrated that SPT positivity to hen's egg at age 1 predicted subsequent development of asthma in adults; however, rhinitis outcomes were not evaluated. Zeiger and Heller<sup>32</sup> demonstrated in a dietary intervention trial that SPT positivity to egg by 12 months of age was associated with asthma, AR, and any atopic disorder at age 7 years. Kulig et al<sup>33</sup> showed that increased serum specific IgE levels to egg in the first 2 years increased the likelihood of AR in 5-year-old children from atopic families. Thus our findings are consistent with previous studies of atopic children.

Spring season of birth significantly increased the risk of AR at age 3 years when compared with winter season of birth. This is consistent with another study showing that seasonal AR was associated with being born during the tree and grass season.<sup>34</sup> We also found that infants with positive SPT responses to tree pollen during the first year of life were at a 5-fold significant increased risk for AR at age 3 years. The effect of early SPT response positivity to aeroallergens on AR has not been assessed in other high-risk birth cohort studies.

Living with multiple siblings in infancy significantly reduced the risk of AR, as well as atopy, but not for the secondary rhinitis outcome, suggesting that this effect was due to atopy. Although living with multiple siblings was protective against rhinitis in population-based epidemiologic studies, it has not been reported in other high-risk birth cohort studies at age 3 years.<sup>35,36</sup>

The central hypothesis of the CCAAPS birth cohort is that early exposure to diesel exhaust pollution, as estimated based on ECAT exposure, increases the risk for allergic disorders in childhood. We found no associations of ECAT exposure in infancy with AR, atopy, or rhinitis at age 3 years. Recently, Morgenstern et al<sup>37</sup> reported that physician-diagnosed hay fever at ages 2 and 6 years

was significantly associated with high PM<sub>2.5</sub> exposure. The conflicting results could be explained by the differences in the study populations and design. The Morgenstern study was population based, skin testing was not used to define AR, and PM<sub>2.5</sub> was used to estimate traffic exposure instead of ECAT, a more precise measure of traffic pollutant exposure.

In summary, this study identified multiple risk factors for early childhood AR in a high-risk cohort. However, these findings might not be generalizable to a population-based sample. For the first time, we demonstrated a protective effect of prolonged breast-feeding in African American children against AR. Infants raised with multiple children in the home were less likely to have AR. The effect of HDE was dependent on exposure level. Infants born in the spring, with positive SPT responses to trees, and with positive SPT responses to milk, eggs, or both were more likely to have AR at age 3 years. These findings indicate that routine skin prick testing of high-risk infants is useful in assessing the subsequent risk of childhood allergy.

**Clinical implications: Skin prick testing identifies infants at risk for AR. Encouraging prolonged breast-feeding could reduce the risk of childhood allergy in African American subjects.**

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**TABLE E1.** Parental skin testing characteristics and the lack of associations with AR, atopy, and rhinitis at age 3 years

	AR		Atopy		Rhinitis	
	No. (%)	P value	No. (%)	P value	No. (%)	P value
No. of parents skin tested						
1	109 (32%)		250 (44%)		196 (35%)	
2	7 (32%)	.97	17 (44%)	.95	14 (36%)	.87
No. of parents with positive SPT responses						
1	115 (32%)		263 (44%)		207 (35%)	
2	1 (50%)	.54	4 (57%)	.71	3 (43%)	.7