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A M E R I C A N C O L L E G E O F
 C H E S T
P H Y S I C I A N S

Sensitization to Aeroallergens and Airway Hyperresponsiveness at 7 Years of Age*

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Study objectives: To investigate the relationship between current and early life factors and airway responsiveness to inhaled methacholine in children with a median age of 7.0 years.

Participants: Study subjects were a subset of a prospective birth cohort of children in the Boston area at high risk for atopy.

Methods: One hundred thirty-one children underwent both skin-prick testing to a panel of aeroallergens and a methacholine challenge test between 6.5 years and 8.8 years of age. Telephone questionnaires were performed at set intervals, and home dust samples were collected in the first year of life.

Results: Of the 131 participating children, 51% (67 patients) had at least one positive skin-prick test response and 28% (37 patients) had airway hyperresponsiveness (AHR) [provocative concentration of methacholine causing a 20% fall in FEV₁ of < 4 mg/mL]. After adjusting for relevant covariates, AHR was strongly associated with sensitization to at least four aeroallergens (odds ratio [OR], 13.41; 95% confidence interval [CI], 3.03 to 59.42). In separate adjusted models, sensitization to cat (OR, 14.73; 95% CI, 3.64 to 59.55), dust mite (OR, 5.13; 95% CI, 1.94 to 13.56), cockroach (OR, 4.00; 95% CI, 1.19 to 13.50), and ragweed (OR, 10.08; 95% CI, 2.31 to 44.10) were significant predictors of AHR. However, no relationship was found with early life exposure to perennial aeroallergens or other perinatal and first-year-of-life factors.

Conclusions: Among young children at risk for atopy, sensitization to specific aeroallergens, but not early life exposures, is associated with increased airway responsiveness.

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Key words: asthma; atopy; bronchial provocation tests; child; cohort studies; immediate hypersensitivity

Abbreviations: AHR = airway hyperresponsiveness; CI = confidence interval; DRS = dose-response slope; OR = odds ratio; PC₂₀ = provocative concentration of methacholine causing a 20% decrease in FEV₁

Airway hyperresponsiveness (AHR) to nonspecific stimuli such as cold air and exercise is one of the cardinal features of asthma. Among children with asthma, higher levels of airway responsiveness to

histamine or methacholine correlate with disease severity, presence of symptoms, and level of treatment required to control symptoms.¹ However, little is known about predictors of AHR in young children, especially those at high risk for asthma and other atopic diseases. A positive association between total

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serum IgE and airway responsiveness has been found in both adults and children.^{2–6} AHR has also been shown to be highly correlated with atopy (positive skin test or radioallergosorbent test results)^{7–13}; however, most of these studies are in older children and adults and are not prospective studies of high-risk birth cohorts in which one can examine the effect of early life exposures and other factors. In addition, none of the studies in young children identify that specific aeroallergen sensitizations are associated with increased risk of AHR.

Sensitization to specific allergens may be important and may vary regionally. AHR has been shown to be associated with sensitization to various allergens (including house dust mite, cat, and Timothy grass) in children ≥ 10 years of age and adults.^{14–19} In addition, AHR has been shown to be correlated with other atopic diseases such as allergic rhinitis^{20,21} and food allergy.²²

One of the primary purposes in the seventh year of the Epidemiology of Home Allergens and Asthma study was to examine the association between allergen sensitization and airway responsiveness at 7 years of age and to assess whether early life exposures and other factors influenced this relationship. This is one of the few prospective birth cohort studies to look at this association at such an early age. Children in our study underwent skin-prick testing to panel of 13 aeroallergens, which is a larger panel of aeroallergens than used by most other studies. In addition, we also had extensive data on both perinatal and early life factors, as well as current symptoms and exposures.

MATERIALS AND METHODS

Study Participants

Five hundred five infants with a history of asthma or allergies in at least one parent were recruited within 48 h of birth between September 1994 and August 1996 in the metropolitan Boston area. Inclusion and exclusion criteria have been detailed previously.²³ In brief, eligibility criteria included a history of asthma, allergies, or hay fever in either parent; maternal age ≥ 18 years of age, maternal ability to speak English or Spanish, and residence within Route 128, a highway that encircles the Boston metropolitan area. Newborns were considered ineligible for enrollment if they were born prematurely (< 36 weeks gestation), admitted to the neonatal ICU, or had a congenital anomaly. Seven children were unavailable for follow-up within the first 4 months and were excluded from further analysis. A further 58 children were excluded because of incomplete follow-up by 6.5 to 7.5 years of age. Permission for allergy skin-prick testing and methacholine challenge testing of the remaining 440 children (87% of the original birth cohort) was sought at the laboratory visit between the ages of 6.5 and 7.5 years. Telephone questionnaires eliciting data on symptoms and diagnosis of atopic diseases as well as allergen exposures were administered every 2 months for the first 2 years of life and then every 6 months thereafter. Dust samples collected

from the child's household in the first year of life were assayed for the presence of allergens, including Fel d 1, Der p 1, Der f 1, and Bla g 1 and 2. Methods for the collection and processing of dust samples and assay of allergens have been previously detailed.²⁴ Informed consent was obtained from the parents of participating children. The study was approved by the Institutional Review Board of the Brigham and Women's Hospital.

Methacholine Challenge Testing

Parents were instructed to hold, if possible, all of their child's medications that might interfere with testing (*eg*, β -agonists, inhaled corticosteroids, leukotriene antagonists, and antihistamines) for 72 h prior to the methacholine challenge. In addition, subjects were to avoid products containing caffeine (*eg*, coffee or cola drinks) on the day of the test. The challenge was only performed if the child had not had an upper respiratory infection within 2 weeks of testing, had not used oral steroids within 4 weeks of testing, and had not used a short-acting bronchodilator within 4 h and a long-acting bronchodilator within 12 h of the test.

First, baseline spirometry was performed using a spirometer (Spiro232; Morgan Scientific; Haverhill, MA). If the child's FEV₁ was $< 70\%$ of predicted, no further testing was done. Otherwise, the child was administered a 1.5-mL dose of saline solution via a PDS Koko Dosimeter (PDS1-414400; Ferraris Respiratory; Louisville, CO), and spirometry was repeated. Again, if the child's FEV₁ was $< 70\%$ of predicted, no further testing was done. Otherwise, the methacholine challenge proceeded using the five-breath dosimeter method and a dosing schedule of 0.025, 0.25, 2.5, 10, and 25 mg/mL of methacholine (the protocol used was similar to that outlined in the 1999 American Thoracic Society guidelines for methacholine and exercise challenge testing).²⁵ The test was terminated when the child's FEV₁ fell by $\geq 20\%$ from the post-saline solution FEV₁ or the top dose was reached, whichever occurred first. Children who reached the top dose without a $\geq 20\%$ fall in FEV₁ were considered nonresponders.

Allergy Skin-Prick Testing

Skin testing was performed on the volar aspect of the lower arms. Parents were instructed to withhold antihistamines for 72 h prior to the test. No skin testing was performed if the child received antihistamines within 72 h of the scheduled test. Pricks were made using the Multi-Test II system (Lincoln Diagnostics; Decatur, IL). Subjects were tested to the following allergens: dust mites (Der p and Der f separately); cockroach mix; cat; dog; mouse; tree mix; grass mix; ragweed mix; Alternaria; Aspergillus; Cladosporium; and Penicillium (Hollister-Stier; Spokane, WA; and Greer Laboratories; Lenoir, NC). Glycerinated saline solution and histamine were used as the negative and positive controls, respectively. The wheal was measured 15 min after application. A test was considered positive if the wheal was at least 3 mm greater than the negative control.

Outcome Variables

Methacholine reactivity was determined by calculating both the provocative concentration of methacholine causing a 20% fall in FEV₁ (PC₂₀)²⁵ and the dose-response slope, which is calculated as the two-point slope from baseline to final FEV₁ regardless of the fall in FEV₁.²⁶ In order to be able to include nonresponders in the analysis using PC₂₀ as the outcome variable, PC₂₀ was dichotomized at a cutoff of 4 mg/mL (based on American Thoracic Society guidelines for a positive test²⁵). This

cutoff has also been used in other studies of AHR in childhood.^{12,20,27} By using the dose-response slope, we were able to analyze methacholine reactivity as a continuous variable. Because the dose-response slope was not normally distributed, this was log transformed for analytic purposes.

Predictor Variables

Sensitization to aeroallergens was considered in several ways. First, we examined the level of sensitization (none, vs one to three, vs four or more allergens). We then looked at allergen groupings: pollens, molds, and indoor allergens. Finally, we considered sensitization to individual allergens, including cat, dog, mouse, dust mites, cockroach, ragweed, grass, trees, *Alternaria*, *Aspergillus*, *Cladosporium*, and *Penicillium*.

Additional Covariates

We also examined whether AHR was associated with a physician's diagnosis of asthma, occurrence of wheezing, or nocturnal cough, as well as other atopic diseases such as eczema and allergic rhinitis. In addition, we considered whether AHR at age 7 years might be associated with early life exposures and other factors, which may be related to allergic inflammation or airway development. These variables include cord blood IgE; cat or dog in the home at 2 to 3 months of age; specific allergen levels in the home at 2 to 3 months of age (using cutoffs of 1 $\mu\text{g/g}$ of dust for Fel d 1 in the family room, 2 $\mu\text{g/g}$ of dust for Der p/f in the family room, and 0.05 U/g of dust for Bla g 1/2 in the kitchen); parental history of asthma; family income; gestational age; birth weight; ponderal index ([birth weight in grams] $\times$ 100/[birth length in cubic centimeters]); and lower respiratory tract illness; smoking in the home (maternal, paternal, or other); breast feeding; and daycare attendance during the first year of life.

Adjusted Analysis

Gender and race were controlled for in the adjusted analyses, as these are factors that have been shown to be associated with asthma in numerous studies. Other variables included in the adjusted analyses were family income and parental history of asthma, which have been shown to be associated with development of asthma^{28,29}; age and height, which are both important factors that influence FEV₁ and have also been shown to effect airway responsiveness in children³⁰; and prechallenge FEV₁.³¹ We also controlled for specific allergen levels in the home and presence of a pet in the home, when relevant.

Statistical Methods

Statistical software (SAS Version 8; SAS Institute; Cary, NC) was used to evaluate univariate associations between the predictor variables and the outcomes of interest and to adjust for potential confounders. Univariate associations between categorical predictor variables and outcomes were first examined with 2×2 contingency tables. Both linear and logistic regression analyses were used to study the relationship between allergen sensitization and AHR. Using the dose-response slope (DRS) allowed us to examine the outcome as a continuous variable, including both responders and nonresponders (those who did and did not have a fall in FEV₁ $\geq 20\%$ at any methacholine concentration) in the analysis. However, while linear regression is more powerful, the results were difficult to translate into clinically relevant information. Therefore, we also used logistic regression with an outcome of dichotomized PC₂₀ (cutoff of 4 mg/mL, with the upper group including the nonresponders) to obtain odd

ratios (ORs). Adjusted analyses were performed, controlling for potential confounders as noted above. One extreme outlier with regards to airway responsiveness was excluded from the regression analysis.

RESULTS

Characteristics of Cohort

At a median age of 7.0 years (range, 6.5 to 8.8 years), 146 of the 440 children underwent a methacholine challenge. Of these children, 26 patients (17.8%) were nonresponders; 107 patients (73.3%) were responders; 3 patients (2.0%) did not have an acceptable final FEV₁ maneuver, but were believed to be true responders because of the presence of respiratory symptoms that interfered with the subjects' ability to perform spirometry; 2 patients (1.4%) were thought to have no acceptable final FEV₁ due to fatigue or distraction; and 8 patients (5.5%) terminated the test prematurely due to factors other than a $\geq 20\%$ fall in FEV₁. The latter two groups were excluded from further analysis. Of the 250 children who underwent skin testing, 132 children also performed adequate methacholine challenges. One child had an extreme outlying value for the DRS, even after log transformation, and was thus excluded from further analysis.

Table 1 summarizes the characteristics of the children included in the analysis. Of these 131 children, a high proportion (28%) responded at a methacholine concentration of ≤ 4 mg/mL (19.8% did not respond to the maximum concentration of 25 mg/mL; 9.2% responded at > 16 to 25 mg/mL; 19.8% responded at > 8 to 16 mg/mL; 22.9% responded at > 4 to 8 mg/mL; 21.4% responded at > 1 to 4 mg/mL; and 6.9% responded at < 1 mg/mL). Fourteen children (11%) had been diagnosed with asthma and had a history of wheezing in the past year, and 18 children (14%) had been diagnosed with allergic rhinitis and were symptomatic in the past year. Seventy-six children (58%) were male, and 105 children (80%) were white. Sixty-five percent of children with AHR were atopic, vs 46% without AHR; 79% with current asthma were atopic, vs 48% without symptomatic asthma.

Comparing those children who did and did not participate in methacholine challenge testing, there were no significant differences in the rate of current asthma (12% vs 9%, $p = 0.35$); race (79% vs 74% were white, $p = 0.17$); gender (56% vs 53% were male, $p = 0.50$); or parental history of asthma (48% vs 54%, $p = 0.22$). Those with a family income $< \$50,000$ at birth were less likely to participate in methacholine challenge testing than those with higher family incomes (20% vs 31%, $p = 0.02$). There was no difference in rate of sensitization to at

Table 1—Subject Characteristics

Variables	Nonresponders, No. (%) [*]	Responders, No. (%)	
		PC ₂₀ ≥ 4 mg/mL	PC ₂₀ < 4 mg/mL
Asthma/wheeze [†]			
Yes	1 (3.8)	7 (10.3)	6 (16.2)
No	25 (96.2)	61 (89.7)	31 (83.8)
Allergic rhinitis [‡]			
Yes	3 (11.5)	8 (11.8)	7 (18.9)
No	23 (88.5)	60 (88.2)	30 (81.1)
Parental asthma [§]			
Yes	10 (38.5)	30 (44.8)	23 (54.8)
No	16 (61.5)	37 (55.2)	19 (45.2)
Sex			
Male	16 (61.5)	38 (55.9)	22 (59.5)
Female	10 (38.5)	30 (44.1)	15 (40.5)
Race			
White	22 (84.6)	51 (75.0)	32 (86.5)
Other	4 (15.4)	17 (25.0)	5 (13.5)
Income [¶]			
< \$50,000	6 (23.1)	14 (21.2)	5 (14.3)
≥ \$50,000	20 (76.9)	52 (78.8)	30 (85.7)

^{*}Nonresponders are those who did not have a > 20% drop in FEV₁ from baseline at any methacholine dose (top dose was 25 mg/mL).

[†]Ever diagnosed with asthma and any history of wheeze in the past year at age 7 years.

[‡]Ever diagnosed with allergic rhinitis or hay fever and any history of allergic nasal and/or eye symptoms in the past year at age 7 years.

[§]Either parent ever receiving a diagnosis of asthma (one subject missing data).

^{||}Black, Hispanic, Asian, and other.

[¶]Family income at enrollment (four subjects missing data).

least one aeroallergen (54% vs 48%, $p = 0.59$) or to specific allergens (all $p > 0.05$, data not shown) between the participants and nonparticipants.

Predictors of AHR

Children with the greatest airway responsiveness (upper quartile of the log-transformed DRS) were much more likely to be sensitized to four or more aeroallergens than those in the lower three quartiles ($p = 0.003$, χ^2). In the univariate analysis using logistic regression (Table 2), the odds of AHR was nearly fivefold higher in those who were sensitized to four or more allergens vs none. While the trend was toward greater airway responsiveness in those sensitized to one to three allergens compared to none, this difference was not statistically significant. As a group, both pollen allergens (trees, grass, and ragweed) and indoor allergens (cat, dog, mouse, dust mites, and cockroach) were associated with AHR. Molds (*Alternaria*, *Aspergillus*, *Cladosporium*, and *Penicillium*), however, were not found to be associated with AHR, although far fewer children were sensitized to molds than indoor or pollen allergens.

Looking at individual allergens, sensitization to cat, dust mite, and cockroach allergens all were associated with AHR (Table 2), and these relationships remained significant despite adjusting for specific allergen exposure in the home. A strong rela-

tionship was also seen between ragweed sensitization and AHR, with a greater than sevenfold increased odds of AHR in children with a positive skin-prick test response to ragweed. We did not see a significant association between AHR and sensitization to dog, mouse, trees, grass, or any of the molds (data not shown). No association was seen between AHR and presence of cat or dog in the home at the initial visit, cat as a pet currently, or level of allergen in the home in the first year of life (Fel d 1 in the family room, Der p 1 and Der f 1 in the family room, and Bla g 1/2 in the kitchen). Nor was current cat exposure associated with AHR among those sensitized to cat ($p = 1.00$, Fisher exact test).

We found no significant relationship between AHR and age, height, baseline FEV₁, or percentage of predicted FEV₁. Race and gender were not associated with AHR, nor were any of the socioeconomic variables, including family income. Furthermore, perinatal and first-year-of-life factors including birth weight, ponderal index, gestational age, cord blood IgE, daycare attendance, breastfeeding, tobacco smoke exposure, and lower respiratory tract infection were not associated with AHR (data not shown). While there was a trend toward a positive association between AHR and current asthma (AHR was present in 43% of children with asthma vs 26% of children without asthma), this relationship was

Table 2—Relationship Between Aeroallergen Sensitization and AHR (Logistic Regression)*

Aeroallergen Sensitization (Covariates)†	PC ₂₀ , No. (%)		Univariate Analysis		Adjusted Analysis§	
	< 4 mg/mL	≥ 4 mg/mL‡	OR (95% CI)	p Value	OR (95% CI)	p Value
All aeroallergens						
No positive skin-prick test results	13 (36.1)	50 (53.8)	1.00	1.00		
One to three positive test results	13 (36.1)	35 (37.6)	1.43 (0.59–3.45)	0.428	1.85 (0.71–4.84)	0.208
More than three positive test results	10 (27.8)	8 (8.6)	4.81 (1.58–14.62)	0.006	13.41 (3.03–59.42)	0.001
At least one indoor allergen	23 (62.2)	32 (34.4)	3.13 (1.42–6.90)	0.005	4.40 (1.72–11.25)	0.002
At least one pollen allergen	15 (40.5)	21 (22.6)	2.34 (1.03–5.29)	0.042	3.40 (1.30–8.85)	0.012
At least one mold allergen¶	4 (11.1)	12 (12.9)	0.84 (0.25–2.81)	0.782	1.21 (0.33–4.49)	0.773
Cat (Fel d 1)¶#	13 (35.1)	10 (10.6)	4.55 (1.78–11.66)	0.002	14.73 (3.64–59.55)	< 0.001
Dust mites (Der p and/or Der f)#	19 (51.4)	20 (21.3)	3.91 (1.73–8.80)	0.001	5.13 (1.94–13.56)	0.001
Cockroach (Bla g 1 and 2)#	8 (21.6)	7 (7.4)	3.42 (1.14–10.28)	0.028	4.00 (1.19–13.50)	0.025
Ragweed	9 (24.3)	4 (4.3)	7.23 (2.07–25.29)	0.002	10.08 (2.31–44.10)	0.002

*An outlier was excluded from this analysis. Two subjects were missing skin testing data for mouse, tree mix, grass mix, *Penicillium* mix, *Cladosporium*, *Alternaria*, and *Aspergillus*. Four subjects were missing data on initial family income, and one subject was missing data on parental history of asthma.

†Aeroallergens include cat (Fel d 1), dog, dust mites (Der p and Der f), cockroach mix (Bla g 1 and 2), mouse, ragweed mix, tree mix, grass mix, *Penicillium* mix, *Cladosporium*, *Alternaria*, and *Aspergillus*.

‡In this analysis, the PC₂₀ ≥ 4 mg/mL group also included nonresponders (those who did not have a ≥ 20% drop in FEV₁ from baseline at any methacholine dose).

§Adjusted for age, race, gender, height, percentage of predicted FEV₁ at baseline, family income level at initial visit, and parental history of asthma (ever).

||Cat, dog, mouse, dust mites, and cockroach. For the adjusted analysis, also controlled for cat exposure in the home (at time of enrollment and current).

¶*Penicillium* mix, *Cladosporium*, *Alternaria*, and *Aspergillus*.

#For the adjusted analysis, also controlled for level of specific allergen exposure (indoor allergen included cat, cockroach, and dust mites).

nonsignificant. Parental history of asthma had a borderline association with AHR ($p = 0.05$). While there did not appear to be a relationship between maternal asthma and AHR, active paternal asthma was associated with AHR ($p = 0.02$).

The adjusted analyses using logistic regression are summarized in Table 2. After controlling for potential confounders, the odds of AHR increased from fivefold to 13-fold if a child was sensitized to multiple allergens. We found sensitization to pollen allergens to be associated with a threefold-increased risk of AHR and sensitization to indoor allergens carried a fourfold-increased risk of AHR. After adjustment, the odds of AHR were nearly 15 times higher if the child was sensitized to cat and 10 times higher if sensitized to ragweed. Risk of AHR with sensitization to dust mites was increased fivefold. Results for cockroach sensitization were similar to those for dust mites, with odds of AHR elevated fourfold. When we additionally controlled for sensitization to other allergens, sensitization to indoor allergens (OR, 3.82; 95% confidence interval [CI], 1.37 to 10.66), cat (OR, 10.27; 95% CI, 1.92 to 55.09), dust mite (OR, 4.47; 95% CI, 1.30 to 15.34), and ragweed (OR, 6.49; 95% CI, 1.16 to 36.41) remained significantly associated with AHR.

Predictors of DRS

Table 3 presents the results of the univariate and adjusted analyses of the relationship between aller-

gen sensitization and the DRS. The results were similar to that seen in the logistic regression, with the following exceptions. While a strong association was not seen for pollen allergens in the univariate analysis, it did approach borderline significance in the adjusted analysis. The association between current asthma and AHR was highly significant ($p = 0.003$). Parental history of asthma was a positive predictor of AHR ($p = 0.01$). Paternal history of asthma appeared to be more important than maternal history ($p = 0.05$ and $p = 0.25$, respectively). After adjusting for exposure to other allergens, sensitization to indoor allergen, cat, and ragweed remained significant, but dust mite did not (data not shown).

DISCUSSION

In this study, we demonstrated an association between AHR and sensitization to aeroallergens in children 6.5 to 8.8 years of age at high risk for asthma and other atopic diseases. A large proportion of children in this high-risk birth cohort had AHR (> 50% responded to a methacholine concentration of ≤ 8 mg/mL, and 28% responded at ≤ 4 mg/mL). We found that a higher level of atopy, with sensitization to multiple allergens, was a strong predictor of AHR. Furthermore, we have shown that sensitization to specific aeroallergens, including cat, dust mite, cockroach, and ragweed, are important predictors of AHR in children living in the Boston area.

Table 3—Relationship Between Aeroallergen Sensitization and AHR (Linear Regression)*

Aeroallergen Sensitization (Covariates)†	Outcome = Log-Transformed DRS for Methacholine Challenge			
	Univariate Analysis		Multivariate Analysis‡	
	Estimate (SE)	p Value	Estimate (SE)	p Value
All aeroallergens				
No positive skin-prick test results	1.00	1.00		
One to three positive test results	0.229 (0.234)	0.331	0.325 (0.234)	0.168
Three or more positive test results	0.873 (0.327)	0.009	1.163 (0.369)	0.002
At least one indoor allergen§	0.628 (0.215)	0.004	0.689 (0.230)	0.003
At least one pollen allergen	0.372 (0.243)	0.128	0.470 (0.256)	0.069
At least one mold allergen	− 0.205 (0.334)	0.540	− 0.072 (0.357)	0.840
Cat (Fel d 1)§	0.738 (0.279)	0.009	1.275 (0.301)	< 0.001
Dust mites (Der p and/or Der f)§	0.608 (0.232)	0.010	0.657 (0.244)	0.008
Cockroach mix (Bla g 1 and 2)§	0.711 (0.337)	0.037	0.724 (0.348)	0.040
Ragweed mix	1.314 (0.346)	< 0.001	1.366 (0.365)	< 0.001

*One outlier was excluded from this analysis. Two subjects were missing skin testing data for mouse, tree mix, grass mix, *Penicillium* mix, *Cladosporium*, *Alternaria*, and *Aspergillus*. Four subjects were missing data on initial family income and one subject was missing data on parental history of asthma.

†Allergens include cat (Fel d 1), dog, dust mites (Der p and Der f), cockroach mix (Bla g 1 and 2), mouse, ragweed mix, tree mix, grass mix, *Penicillium* mix, *Cladosporium*, *Alternaria*, and *Aspergillus*.

‡Cat, dog, mouse, dust mites, and cockroach. Adjusted for age, race, gender, height, percentage or predicted FEV₁ at baseline, family income level at initial visit, and parental history of asthma (ever).

§For the adjusted analysis, also controlled for level of specific allergen exposure (for 'indoor' included cat, cockroach, and dust mites).

||For the adjusted analysis, also controlled for cat exposure in the home (at time of enrollment and current).

However, we found no association with early life perennial aeroallergen exposure, either for specific allergens levels or presence of a pet in the home. Nor did we find a relationship between AHR and first year of life factors such as daycare attendance, breastfeeding, tobacco smoke exposure, and lower respiratory tract infection.

While low lung function appears to be the major risk factor for transient wheezing in the first 3 years of life, children with persistent wheezing are usually atopic and have a family history of asthma and allergies.^{32,33} In one of the earliest studies from this birth cohort, Gold et al²³ demonstrated that low birth weight, maternal smoking, lower respiratory tract infection, and higher levels of cockroach allergen exposure were predictive of repeated wheeze in the first year of life. While we did not see an association with birth weight and AHR in this analysis, this is likely due to the fact that premature infants were not enrolled in the study. Thus, we likely did not have sufficient power to see any association with birth weight. Indeed, in an analysis of the whole cohort at 6 years, Raby et al³⁴ found that low normal gestational age was associated with asthma. In this study, we demonstrate that sensitization to multiple aeroallergens is a strong predictor of AHR in children 6.5 to 8.8 years of age, over and above baseline lung function and other factors associated with airway size and development. This is consistent with the hypothesis that infants with narrowed or overly compliant airways are more

susceptible to virally induced wheezing, but as the children grow up factors related to allergic inflammation become more important predictors of persistent wheezing, asthma, and AHR.

In our study, the most important allergen sensitizations in predicting risk of AHR were those to cat, dust mite, cockroach, and ragweed. The association between AHR and specific sensitization to cat and dust mite has been demonstrated in European adults aged 20 to 44 years,¹⁹ New Zealand children 13 years of age,¹⁸ and 10-year-old schoolchildren from China.¹⁶ However, it has not been previously shown in such a young cohort. Lau et al¹³ did find an association between wheezing and specific sensitization to dust mite and cat in their cohort of 7-year-old children, but were only able to demonstrate a relationship between AHR and indoor allergens as a group. In that study,¹³ 38% of the original cohort were infants at high risk for asthma; however, the rest of the subjects were drawn from a sample of the general population, and only 6% of their cohort had ever received a diagnosis of asthma by age 7 years (less than half the rate seen in our cohort). There were no details on specific rates of sensitization to dust mite and cat.

In the study by Carter et al,³⁵ an association was seen between dust mite sensitivity and physician-diagnosed asthma but not AHR at 6 to 7 years of age. Their sample size was smaller (only 65 children), and they may not have had sufficient power to detect an association. They also used a higher PC₂₀ cutoff up

to 10 mg/mL to define AHR, which would include some patients with borderline AHR according to the American Thoracic Society guidelines.²⁵ As with study by Lau et al,¹³ their sample of children appears to be less atopic, with only 6 of 65 children (9%) ever receiving a diagnosis of asthma and sensitization rates of 18% (12 of 65 children) to Der f 1 and 21% (14 of 65 children) to Der p 1, compared to 13% of children ever receiving a diagnosis of asthma and 34% sensitized to Der p1 and Der f 1 in our sample from this high-risk cohort.

Sensitization to outdoor allergens has been shown to be associated with allergic rhinitis but not asthma. In our study, a positive skin test result to at least one outdoor allergen appears to predict AHR. This is consistent with previous studies^{20,21} that have shown that those with allergic rhinitis also have higher AHR, even if they have no history of wheezing. Ragweed is a prominent allergen in the Northeast and a major trigger for allergic rhinitis symptoms in the late summer and early fall. Ragweed, rather than grass or tree pollens, is the pollen allergen most strongly associated with AHR in our study.

Maternal, and not paternal, history of asthma appears to predict asthma in young children. Martinez et al³³ noted that children who had early onset persistent wheezing were more likely than children who had never wheezed to have mothers with a history of asthma. In the children in this birth cohort, asthma at 6 years of age was associated with active maternal asthma.³⁴ In the analysis of the siblings of the children in this birth cohort, maternal asthma was found to be a significant predictor of asthma among children < 5 years of age, but both maternal and paternal history of asthma were predictors of asthma among children > 5 years of age.³⁶ Data regarding the importance of parental asthma in determining risk of AHR in the child have been mixed. Our findings show that paternal asthma, but not maternal asthma, is associated with AHR in children 7 years of age. Raby et al³⁷ also found that paternal history of asthma is associated with greater AHR in children with established asthma. However, Kurukulaaratchy et al¹² found that maternal, rather than paternal or sibling, asthma was a more relevant predictor of symptomatic AHR in 10-year-old children. This highlights the fact that asthma and AHR are not the same entity, and we emphasize that our study investigated the predictors of AHR and not asthma, *per se*. Further study into the differential inheritance of AHR is needed.

In the siblings in this cohort at < 5 years of age, exposure to higher levels of cockroach allergen was associated with recurrent asthmatic wheeze³⁸; the sensitization status of these children was not known. The findings were mixed for cat allergen exposure in

the first 5 years of life and the outcome appeared to be dependent on maternal history of asthma. Children whose mothers had asthma were at increased risk for wheezing if they were exposed to a cat or high levels of allergen, whereas such exposures in children whose mothers did not have asthma appeared to be protective, with a decreased risk of wheezing.³⁹ Because of the relatively small sample size of our study, we were not able to examine whether maternal history of asthma modifies the relationship between cat allergen exposure and AHR. In this study, higher levels of home allergens in the first year of life were not related to AHR at 7 years of age, nor was presence of an animal in the home. This may be due to the small sample size and resultant loss of power. However, it is consistent with the few studies^{13,35,40} that have looked at the relationship between allergen exposure in early life and AHR later in childhood. Other studies^{41,42} have shown that current exposure to allergens such as cockroach and dust mites in specifically sensitized children was associated with a current diagnosis of asthma, recurrent wheezing, AHR, and hospitalization with an asthma exacerbation. Exposure to high levels of indoor allergens has even been associated with AHR in nonsensitized atopic asthmatics.⁴³ Our findings and the findings of others noted above^{41,42} suggest that it may be ongoing allergen exposure, rather than just early life exposure, that is an important determinant of AHR. In epidemiologic studies, it is difficult to measure ongoing exposure to allergens, since children may also be exposed outside the home and studies would have to account for parental behaviors that may change indoor allergen levels. While we did not include current allergen levels in this analysis, it is likely that sensitization to allergens is a measure of cumulative exposure to allergens in the environment of the child.

There are several limitations in our study. Because we analyzed a subgroup of the cohort, there was some loss of statistical power, which may have resulted in erroneous null associations. We did see some selection bias in that children from lower income families were less likely to participate in methacholine challenge testing. However, we found no association between initial family income and AHR, and controlling for family income in the adjusted analyses did not alter our findings. In addition, subjects from disadvantaged populations have greater degrees of AHR⁴⁴ and have a greater prevalence of allergic sensitization.⁴⁵ Therefore, it is likely that the results presented here for the association between sensitization and AHR are underestimates of the true relationship. Thus, this selection bias represents a bias toward the null. While the lack of measurement of allergen levels outside of the

home (eg, daycare) may have resulted in misclassification of exposure, we did not see an association between daycare attendance and atopic sensitization. We also recognize that our cohort subjects were selected for their high risk for asthma and allergies. While this may limit generalizability, it is in this high-risk population that the association between AHR and atopy needs to be better understood. Finally, while there is a strong association between AHR and asthma, the two are not equivalent. Some individuals have AHR and do not have overt clinical asthma. In the general pediatric population, the prevalence of asymptomatic AHR is 7 to 8%.^{46,47} While they may be currently asymptomatic, Carey et al⁴⁸ demonstrated that these children have a fourfold increased risk for asthma symptoms developing at a later time. Future longitudinal studies will be important in clarifying the utility of methacholine challenges in determining both the presence of and risk for asthma.

In summary, responsiveness to methacholine is highly prevalent in a cohort at high risk for atopic disease at 6.5 to 8.8 years of age. Sensitization to cat, dust mite, cockroach, and ragweed are important predictors of AHR, while early life exposures do not appear to play a role.

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