Environmental Tobacco Smoke and Interleukin 4 Polymorphism (C-589T) Gene: Environment Interaction Increases Risk of Wheezing in African-American Infants

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Objectives To determine whether infants exposed to environmental tobacco smoke (ETS) having the interleukin 4 (IL-4) or interleukin 13 (IL-13) gene polymorphisms were at increased risk of wheezing.

Study design A birth cohort of 758 infants was evaluated annually by a questionnaire, physical examination, and skin prick testing. DNA samples from 560 children were genotyped for IL-4 C-589T and IL-13 C-1112T. The relationship of ETS exposure and genotype with the outcome of wheezing was analyzed.

Results At the time of evaluation, mean age was 13.4 ± 2.2 months. The prevalence of sensitization was 29%, and wheezing without a cold was 26.2%. The interaction of ETS exposure and the CT/TT genotypes for IL-4 C-589T showed a significant association with wheezing (odds ratio: 10.84; 95% confidence interval: 1.12-104.64, $P = .04$) in African-American infants.

Conclusions In African-American infants with a family history of atopy, the interaction of ETS and IL-4 C-589T demonstrated a 10-fold risk associated with wheezing without a cold. *(J Pediatr 2008;152:709-15)*

Both genetic and environmental factors contribute to the pathogenesis of asthma. Children exposed to environmental tobacco smoke (ETS) have increased asthma exacerbations, wheezing, bronchial hyperreactivity, and impaired lung function.[1-3] Furthermore, maternal smoking during pregnancy has been associated with increased physician diagnosed asthma in children.[3]

Interleukin 4 (IL-4) and interleukin 13 (IL-13) are of particular interest in atopic disease.[4,5] IL-4 influences the generation and regulation of allergic inflammation in asthma.[6] A genetic variant of IL-4, the promoter single nucleotide polymorphism (SNP) C-589T, has been associated with increased IgE levels in families with asthma.[6] The C-589T variant has functional relevance, with increased reporter gene activity in vitro.[6] In several populations, the C-589T variant has been associated with asthma and asthma severity.[7-10] IL-13 also has prominent effects on airway hyperresponsiveness and mucus production.[11] The TT genotype of the IL-13 promoter SNP C-1112T has increased binding of nuclear proteins and decreased inhibition of IL-13 when exposed to anti-CD2 antibody.[12] In several populations, C-1112T has been associated with asthma and serum IgE, a marker of atopic disease.[13,14]

Exposure to ETS can modulate immune responses by affecting cytokine production.[15,16] Increased IL-4 levels in the lungs of mice exposed to ETS have been reported.[17] In human beings, IL-4 levels were found to be increased in smokers and in nasopharyngeal aspirates of those exposed to ETS.[15,16] Increased IL-13 levels were reported in the blood and nasopharyngeal aspirates of patients exposed to ETS.[15,18,19] The increased IL-4 and IL-13 cytokine levels reported in smokers suggest a potential gene:environment interaction.

The objective of this study was to determine whether infants exposed to high levels of ETS who possessed IL-4 or IL-13 gene polymorphisms were at increased risk for the

AA	African-American	IL-4	Interleukin-4
CCAAPS	Cincinnati Childhood Allergy and Air Pollution Study	IL-13	Interleukin-13
		OR	Odds ratio
CI	Confidence interval	SNP	Single nucleotide polymorphism
ETS	Environmental tobacco smoke	SPT	Skin prick test
HWE	Hardy-Weinberg equilibrium		

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Supported by NIEHS Grants ES10957 and ES11170 and NIAID Grant T32-AI60515-02. M. Villareal has consultant arrangements with Aventis, has stock or other equity ownership with Pfizer, and is on the speakers' bureaus for AstraZeneca, UCB Pharma, Pfizer, Aventis, and GlaxoSmithKline.

Submitted for publication Feb 21, 2007; last revision received Jul 30, 2007; accepted Oct 15, 2007.

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0022-3476/$ - see front matter

10.1016/j.jpeds.2007.10.011

development of wheezing in the first year of life. We hypothesized that the genotypes of IL-4 C-589T and IL-13 C-1112T SNPs could significantly modify the effect of environmental tobacco smoke exposure on wheezing at 1 year of age.

METHODS

Source of Data and Sample Size

The Cincinnati Childhood Allergy and Air Pollution Study (CCAAPS) is an ongoing longitudinal birth cohort study whose aim is to determine whether early exposure to traffic pollutants affects development of allergen sensitization and allergic disorders. Children in CCAAPS were recruited on the basis of estimated exposure to diesel exhaust particulates, defined as exposed (<400 m from a major highway) or unexposed (>1500 m from a major highway).[20]

Children with at least 1 confirmed atopic parent (symptomatic with a positive skin prick test result [SPT]+) were enrolled to generate a cohort at high risk for development of allergic disorders.[21] Racial status was defined as African-American (AA) or non-African-American (non-AA) (96% white), on the basis of parental report of infant race. The University of Cincinnati Institutional Review Board approved the study and informed consent.

In the study design phase, the sample size of the study cohort was determined on the basis of careful power analysis. With a baseline rate of wheezing without a cold of 26.2%, genotype frequency of 35% to 40%, exposure to high ETS levels of 25%, a genetic RR of 3, and a fixed cohort size of approximately 750, the power of the study to detect a statistically significant effect ($P = .05$) was calculated to be 92%.[22,23]

Data Collection

All infants were evaluated on an annual basis by a medical and environmental questionnaire administered to the accompanying parent, by physical examination by a clinician, and by SPT to 15 common aeroallergens and 2 foods, milk and egg white (ALK-Abelló, Inc., Round Rock, Texas). The questionnaire was adapted from one that has been validated in the International Study of Allergies and Asthma in Children.[24,25]

Definition of Wheeze

Parental report of infant wheezing without a cold was the outcome of interest.[21,26,27] At the first annual visit, parents received a personal interview by a health care professional regarding their infant's respiratory health. The interview included questions regarding the number of episodes of wheezing observed by the parents with and without a cold, "In the past 12 months, have you ever noticed your child wheezing? If yes, about how many days have you noticed your child wheezing?" and "About how many episodes of wheezing occurred after a cold or infection?"

Exposure Definitions

The annual evaluation included questions regarding parental smoking status, quantity smoked, and average daily length of time that the infant was exposed to tobacco smoke. To estimate total ETS exposure for each infant, the total number of cigarettes smoked daily by each smoker living in the infant's home was added as previously published.[28] ETS exposure was categorized as high (≥20 cigarettes/d), low (1 to 19 cigarettes/d), or none (no cigarette exposure) over the first year of life.

Traffic exposure was defined as unexposed, exposure to moving traffic, or exposure to stop-and-go traffic as previously reported.[20] Exposure to moving traffic was defined as living within 400 m of an interstate or within 100 m of a state route with a speed limit ≥50 miles/h. Exposure to stop-and-go traffic was defined as living within 100 m of a bus route or within 100 m of a state route with a speed limit <50 miles/h. Exposure to endotoxin was determined by dust sampling at an average of 8 months of age and analysis performed as previously described (limulus amebocyte lysate test; Associates of Cape Cod Inc, Falmouth, Mass).[24,29]

DNA Collection and Genetic Analysis

At the initial 12-month visit, buccal swab samples were collected from infants after obtaining parental consent. Genomic DNA was extracted using the ZR Genomic DNA II Kit (Zymo Research Corp, Orange, Calif). The DNA samples were genotyped for the IL-4 C-589T (rs2243250) and IL-13 C-1112T (rs1800925) polymorphisms with the Roche LightTyper platform, Roche Diagnostics, Indianapolis, IN, (based on fluorescence resonance energy transfer) and specific fluorescent probes. Primers and probes were designed using Roche's LightCycler Probe Design Software 2.0, Roche Diagnostics (Appendix I; available at www.jpeds.com). A 10% random resampling was performed to assess the accuracy of the genotype data and revealed greater than 96% agreement.

Data Analysis

The data analysis was performed with SAS software (version 9.1 for Windows; SAS Institute Inc, Cary, NC). Genotype frequencies for IL-4 C-895T and IL-13 C-1112T were evaluated for Hardy-Weinberg equilibrium (HWE) with a χ^2 analysis. If the total study sample was not in HWE, the analysis was then stratified by race to minimize confounding by admixture.[30,31]

Factors potentially associated with wheezing without a cold were initially evaluated by univariate logistic regression. The interaction of ETS exposure (high vs low/none) and genotype (wild type vs at least 1 mutant allele) was included in the analysis, as per SAS syntax.[32] Any factor with a P value ≤ .15 by the univariate analysis was considered for inclusion in the multiple logistic regression model. Once all clinically and statistically relevant factors were identified for inclusion in the multiple logistic regression, "backward elimination" was performed to remove nonsignificant variables.[33] The

Table I. Demographic and Personal Characteristics of the Study Sample

Characteristic	Number (%)			Total Number*
	Non-AA	AA	All	
Child's mean age at first visit	13.3 ± 2.2 months	13.7 ± 2.3 months	13.4 ± 2.2 months	N/A
Infant race	607 (80.6)†	146 (19.4)		753
Income <$40,000	139 (23.6)	112 (80)	251 (34.5)	728
Male sex	329 (54.2)	81 (55.5)	410 (54.5)	753
Infant with at least 1 positive SPT	161 (28.2)	43 (33.6)	204 (29.2)	699
Paternal asthma	69 (11.8)	16 (12)	85 (11.9)	714
Maternal asthma	129 (21.8)	36 (26.1)	165 (22.6)	730
Dog ownership	268 (49.4)	32 (27.1)	300 (45.4)	661
Cat ownership	260 (47.5)	22 (18.6)	282 (42.4)	665
No pet ownership	163 (29.2)	85 (71.4)	248 (36.6)	678
Breast fed at least 1 week	415 (73.2)	61 (48.8)	476 (68.8)	692
Breast fed at least 4 months	304 (53.6)	37 (29.6)	341 (49.3)	692
High ETS exposure (≥ 20 cigarettes/day)	86 (14.7)	14 (9.7)	100 (13.7)	731
Moving traffic exposure	140 (27.5)	27 (28.1)	167 (27.6)	605
Stop-and-go traffic exposure	60 (11.8)	29 (30.2)	89 (14.7)	605
Wheezing without a cold‡	142 (25.0)	41 (31.8)	183 (26.3)	697
Bronchodilator use in past year	40 (7.0)	22 (15.3)	62 (8.7)	714
Prednisone use in past year	16 (2.8)	7 (4.9)	23 (3.2)	714

*Number of infants may vary due to missing information.
†Of the non-AA infants, 96% were white.
‡No significant difference in wheezing % between racial groups ($P = .12$).

analysis was adjusted for potential factors previously reported to be associated with early wheezing including sex, pet ownership, income (<$40,000 or ≥$40,000), and proximity to traffic, as well as other clinically relevant factors by examining changes in odds ratios with the addition of the confounders to the final model. Unadjusted and adjusted odds ratios (OR) and 95% confidence intervals (CI) were calculated. A 2-sided P value of <.05 was considered statistically significant.

RESULTS

Demographics of the Study Sample

Of the 758 infants enrolled in the CCAAPS study, 710 (94%) returned for the 12-month visit and 560 of 710 (79%) agreed to provide a DNA sample. The demographic and personal characteristics of the study sample are summarized in Table I. The study sample was diverse, with good regional representation of AA infants (19.4%) and lower income households (34.5%). Of the non-AA infants, 96% were white. The mean age (±SD) of the infants at the initial visit was 13.4 ± 2.2 months. Consistent with the selection criteria for the study to create a cohort at high risk for atopic disease, at least 1 parent was SPT+, and there was a high prevalence of asthma reported by the mother (22.6%) or the father (11.9%). The rate of infant sensitization on SPT was 29.2% to at least 1 of 17 allergens. There was no significant difference in the rate of wheezing without a cold between the racial groups (non-AA: 25.0%, AA: 31.8%, $P = .12$). During year 1, 62 (8.7%) reported bronchodilator use, and 23 (3.2%) reported prednisone use.

Exposures of the Study Sample

Several exposures that have been reported to be associated with infant wheezing were measured and summarized in Table I. Pet ownership was common in the study sample; 300 (45.4%) reported dog ownership and 282 (42.4%) reported cat ownership. Most infants (476 [68.8%]) in the study had been breast fed for at least 1 week and 341 (49.3%) were breast fed for 4 months. Exposure to moving traffic was present in 167 (27.6%) and to stop-and-go traffic in 89 (14.7%) infants. High ETS exposure (≥ 20 cigarettes/day) was reported for 100 (13.7%) infants.

IL-4 and IL-13 Promoter Genotypes of the Study Sample

For the IL-4 C-589T SNP, 428 samples were available with both genotypes and complete phenotype data. For the IL-13 C-1112T SNP, 560 samples were available with both genotypes and complete phenotype data. There were no significant differences in income, child sex, or ETS exposure between those who agreed to be genotyped, those who did not, and those providing baseline data regardless of genotyping status. The genotypes were evaluated for HWE (Table II). For the total study sample, the genotypes for the IL-4 SNP and IL-13 SNP were not in HWE, indicating possible population substructuring due to admixture. Further analyses were then stratified by race to minimize the effects of admixture.

Among 65 AA infants, 29 (44.6%) had 1 mutant allele (CT) and 26 (40.0%) had 2 mutant alleles (TT) for IL-4 C-589T. For IL-13 C-1112T, 38 of 76 (50.0%) had 1 mutant

Table II. Genotype Frequencies of the IL-4 C-589T and IL-13 C-1112T SNPs by Racial Categories

	Race		
	All	**Non-AA**	**AA**
IL-4 C-589T SNP Genotype			
CC	278 (65.0%)	268 (73.8%)	10 (15.4%)
CT	107 (25.0%)	78 (21.5%)	29 (44.6%)
TT	43 (10.1%)	17 (4.7%)	26 (40.0%)
Total	428	363	65
HWE (χ^2 value)	No (33.9)	No (11.3)	Yes (0.16)
IL-13 C-1112T SNP Genotype			
CC	298 (60.7%)	273 (65.8%)	25 (32.9%)
CT	151 (30.8%)	113 (27.2%)	38 (50.0%)
TT	42 (8.5%)	29 (7.0%)	13 (17.1%)
Total	491	415	76
HWE (χ^2 value)	No (23.9)	No (11.7)	Yes (0.05)

allele (CT) and 13 (17.1%) had 2 mutant alleles (TT). Among the AA infants, both the IL-4 C-589T and IL-13 C-1112T genotypes were in HWE (Table II).

Among 363 non-AA infants, 78 (21.5%) had 1 mutant allele (CT) for IL-4 C-589T and 17 (4.7%) had 2 mutant alleles (TT). For IL-13 C-1112T, 113 of 415 (27.2%) had 1 mutant allele (CT), and 29 (7.0%) had 2 mutant alleles (TT). Among the non-AA infants, neither genotype was in HWE most likely because of population substructure.

Univariate Analysis

For the analyses, ETS exposure was recategorized as high (≥ 20 cigarettes/day) or low/none (0-19 cigarettes/d) on the basis of equivalent rate of wheezing and null effect for the low and none groups (28% in AA and 24% in non-AA). Given the genetic results, the univariate analysis with the outcome of wheezing without a cold at age 1 was stratified by race (Table III). Among the AA infants, 3 factors were statistically significant ($P \leq .05$). Income <$40,000 was associated with an increased odds of wheezing (OR 3.49; 95% CI 1.12-10.88; $P = .03$), as was SPT positivity to any pollen (OR 3.75; 95% CI 1.23-11.39; $P = .02$). Neither genotype alone nor ETS exposure alone was associated with wheezing. However, the interaction of high ETS exposure with IL-4 C-589T genotype (CT or TT) showed a significant 10-fold association (OR 10.00; 95% CI 1.08-92.49; $P = .04$). Another approach to evaluating the data among the AA infants was to examine the proportion with wheezing without a cold on the basis of genotype and ETS exposure (Figure, *A*). Among the AA infants, high ETS exposure conferred significant risk for wheezing only in infants with the CT or TT IL-4 C-589T genotypes ($P = .01$). No statistically significant difference was seen with ETS exposure in the AA infants with the CC IL-4 C-589T genotype.

Among the non-AA infants (Table III), only 1 factor was statistically significant in the univariate analysis. History of paternal asthma was strongly associated with increased odds of wheezing (OR 2.21; 95% CI 1.27-3.86; $P < .01$). Given the overwhelming effect of paternal asthma history in non-AA infants, a subgroup analysis was performed on non-AA infants with no history of paternal asthma (n = 494) (Appendix II; available at www.jpeds.com). Among this subgroup, 2 factors were significant in the univariate analysis; higher average endotoxin exposure showed an inverse relationship with wheezing (OR for 100 unit increase 0.77; 95% CI 0.61-0.96, $P = .021$), and cat ownership was associated with increased odds of wheezing (OR 1.86; 95% CI 1.14-3.03; $P = .013$). A further subgroup analysis was performed among the non-AA infants, evaluating only those infants with at least 1 positive SPT result (n = 153) (Appendix II). Among this subgroup, 2 factors were significant in the univariate analysis. Cat ownership and SPT result positive to any mold were associated with increased odds of wheezing (OR 2.83; 95% CI 1.22-6.59; $P = .016$; OR 2.26; 95% CI 1.01-5.08; $P = .048$).

Results of the Multiple Logistic Regression

Multiple logistic regression was performed, including factors identified in the univariate analysis (Table III and Appendix II) and controlling for sex, pet ownership, income (<$40,000 or ≥$40,000), and proximity to traffic. The analysis was stratified by race, and subgroup analysis was performed as in the univariate analysis. For AA infants, as hypothesized, the interaction of high ETS exposure with IL-4 C-589T genotype (CT or TT) remained significantly associated with wheezing in the multiple logistic regression (OR 10.84; 95% CI 1.12-104.64; $P = .04$) (Figure, *B*). SPT result positive for any pollen was also found to be significantly associated with wheezing (OR 6.65; 95% CI 1.15-38.44; $P = .04$).

Among the non-AA infants, paternal asthma history remained the dominant effect (OR 2.13; 95% CI 1.13-4.04; $P = .02$). Average endotoxin exposure, however, approached but did not reach statistical significance for association with wheezing (OR 0.84; 95% CI 0.70-1.02); $P = .08$).

In the subgroup analysis of non-AA infants without a history of paternal asthma, 2 factors were significant in the multiple logistic regression. Increased average endotoxin exposure was associated with a decreased odds of wheezing (OR for 100 unit increase 0.79; 95% CI 0.63-0.997; 0.05). In contrast, cat ownership was associated with an increased odds of wheezing (OR 1.74; 95% CI 1.00-3.01; $P = .05$).

In the subgroup analysis of non-AA infants with at least 1 positive SPT result, 2 factors were significant. Infants with a paternal history of asthma had an increased odds of wheezing (OR 5.94; 95% CI 1.35-26.06; $P = .02$). Cat ownership was also associated with an increased odds of wheezing (OR 4.08; 95% CI 1.08-15.41; $P = .04$).

DISCUSSION

This study is unique in examining these particular cytokine promoter variants (IL-4 C-589T and IL-13

Table III. Univariate Analysis and Multiple Logistic Regression of Wheezing Without a Cold at Age 1

Factor	Univariate Analysis		Multiple Logistic Regression	
	P Value	OR (95% CI)	*P* Value	OR (95% CI)
Results for AA infants (n = 129)				
Income <$40,000	.03*	3.49 (1.12-10.88)*	.20	3.08 (0.55-17.21)
Pollen + SPT	.02*	3.75 (1.23-11.39)*	.04*	6.65 (1.15-38.44)*
IL-4 C-589T (CT/TT) × High ETS exposure	.04*	10.00 (1.08-92.49)*	.04*	10.84 (1.12-104.64)*
Results for non-AA infants (n = 568)				
Paternal asthma	.005*	2.21 (1.27-3.86)	.02*	2.13 (1.13-4.04)
Average endotoxin exposure (OR for 100 unit increase)	.10	0.86 (0.73-1.03)	.08	0.84 (0.70-1.02)

Multiple logistic regression analyses controlled for sex, pet ownership, income (<$40,000 or ≥$40,000), and proximity to traffic.
*$P \leq .05$.

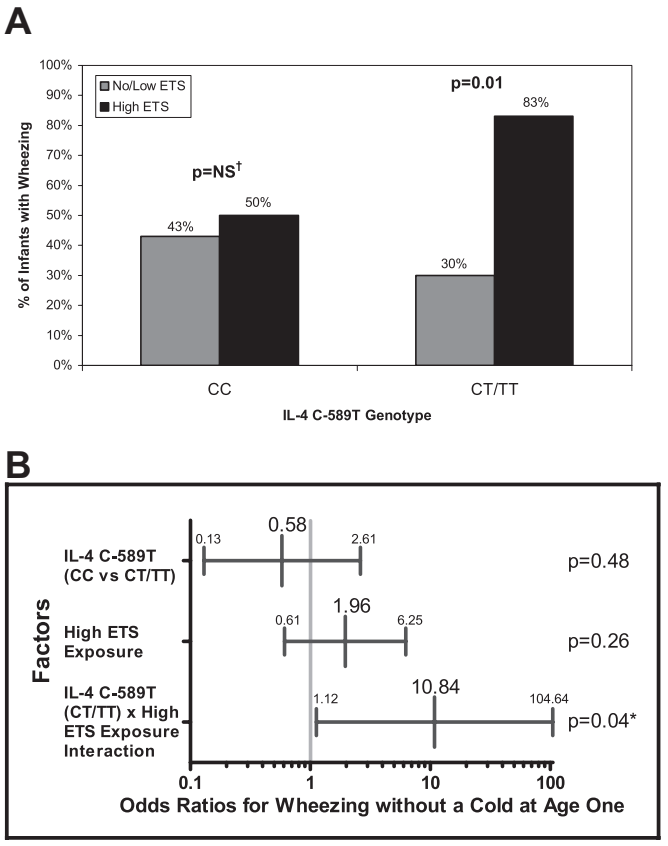

Figure. Interaction of IL-4 C-589T genotype and ETS exposure on wheezing without a cold at age 1in AA infants. **A,** Frequency of wheezing without a cold by IL-4 C-589T genotype and ETS exposure. †Given the small sample size for the CC group, results for this group should be interpreted with caution. **B,** Interactive effects of IL-4 C-589T genotype and ETS exposure on wheezing without a cold. For AA infants, the 65 with genotype data were included in the analysis. For each factor, numbers are odds ratio, 95% confidence interval, and *P* value. **P* value is for the OR of 10.84.

C-1112T)-ETS exposure interaction in infants. Our study demonstrates that among AA infants, although ETS exposure and IL-4 SNP genotype alone do not have a significant effect in this population, the interaction between high ETS exposure and the CT or TT genotypes for IL-4 C-589T significantly increased the odds for wheezing without a cold at age 1. Differential effects of ETS exposure were not seen with the CC genotype for IL-4 C-589T, although the sample size is small, which limits conclusions that can be drawn in this subgroup. The same effect was not seen for IL-13 C-1112T genotype and ETS exposure.

From a public health perspective, our findings may explain some of the disparate burden of asthma among AA children. The asthma attack prevalence is 44% higher for AA children compared with white children.[34] In this cohort, the smoking rate was even higher than the estimated smoking rate for US adults in 2004 of 20.9%.[35] Thus a first risk factor for the AA infants was ETS exposure, with a smoking rate of 33.3% for the AA households and 26.9% for the non-AA households. Given the increased exposure to ETS in the AA infants and the known health effects of ETS exposure on early respiratory health,[1-3] it is therefore not surprising that the AA infants had increased but nonsignificant odds of wheezing without a cold. A second risk factor is the IL-4 C-589T genotype. The genetic differences between the 2 racial groups were striking. It has been reported that the frequency of the alternate genotypes CT or TT for the SNP varies greatly by ethnic group.[10] The IL-4 C-589T genotype frequencies in non-AA infants in this study were similar to what has been reported in the literature.[7,36] In African-Americans, the mutant allele frequencies are much higher (T: 0.54-0.67; C: 0.33-0.46) as compared with in whites.[7,36] These reported frequencies are in agreement with our data. Given that the IL-4 C-589T variant has been associated with asthma and asthma severity.[7-10,37] AA infants having this polymorphism appear to have an increased risk at baseline, but only in the presence of exposure to ETS. In our study, neither ETS exposure nor IL-4 C-589T genotype alone was associated with wheezing. The 10-fold effect of the high ETS exposure × IL-4 C-589T CT/TT genotype interaction is striking and was independent of other risk factors. This 10-fold risk is indicative of a synergistic effect that is far greater than the additive effects of either factor alone (Figure, *B*). It should be noted, however, that the confidence intervals about this 10-fold risk were wide, related to the relatively small number of AA children residing in the presence of

household smoking. Thus this finding needs to be tested in other studies. On the basis of our findings, however, AA children with the mutant alleles of IL-4 C-589T and who were exposed to ETS had a disparate burden of early wheezing.

Among the non-AA infants, different effects were seen. High ETS exposure, IL-4 SNP genotype, IL-13 SNP genotype, and the gene:environment interaction were not significant. In contrast to the AA infants, history of paternal asthma was found to have more than a 2-fold increase in wheezing in non-AA infants by multiple logistic regression. Among non-AA infants with no history of paternal asthma, higher average endotoxin exposure was associated with decreased odds of wheezing, consistent with results with other wheezing outcomes in this cohort.[26] In contrast, cat ownership increased the odds of wheezing. Among non-AA infants with at least 1 positive SPT, both a history of paternal asthma and cat ownership increased the odds of wheezing.

Previous studies have reported that a history of parental asthma, either maternal[38] or paternal,[39] is a risk factor for the development of both wheezing and asthma in children. In this study, only paternal asthma in non-AA infants was a significant risk factor for wheezing without a cold. It is therefore possible that paternal asthma history may be a confounder for some other genetic polymorphism in non-AA infants.

Another possible explanation for this parental difference is epigenetics, stable alterations in gene expression potential that arise during development and cell proliferation.[40] Epigenetic mechanisms may start to explain some of the discordant results in studies of asthma, particularly differences in age of onset, severity of disease, environmental effects, gene:environment interactions, and parent-of-origin effects.[40] Gene expression can be modified by changes in chromatin structure such as DNA methylation, remodeling of nucleosome structure, and covalent modification of nucleosome histones.[41] Histone modifications and DNA methylation status have both been studied in the regulation of IL-4 expression. The basal level of methylation of the IL-4 gene strongly influences early IL-4 expression in naïve T cells.[41]

Our results indicating an inverse association between endotoxin levels at 8 months of age and wheezing during infancy are consistent with results with other wheezing outcomes in this cohort.[26] However, these results were obtained in a post-hoc subgroup analysis and therefore should be interpreted with caution, especially given that other studies have shown an increased risk of wheezing with increased endotoxin exposure.[42,43] Although pet ownership had been associated with a decreased risk of early wheeze in some studies[44,45] and has been shown to increase endotoxin levels that might therefore provide some protection from the development of atopic disease,[46-48] our results indicate that cat ownership throughout the first year of life is an independent risk factor for early wheezing. This finding is consistent with other reports indicating an increased risk of wheezing with increased Fel d1 exposure.[49,50]

One limitation of studying wheezing in the first year of life is that it may not be a good predictor of later development of childhood asthma.[51,52] Several patterns of childhood wheezing have been identified, including transient early wheezing, late onset wheezing, and persistent wheezing.[51,52] In 1 study, 59% of those who had wheezing before age 3 years were not still wheezing at age 6 years.[52] In contrast, infants with persistent wheezing are reportedly more likely to have physician diagnosed asthma at age 6.[52] As the present cohort ages and allergic disease phenotypes are confirmed, we will be able to further investigate the relationship between the IL-4 C-589T SNP, ETS exposure, and asthma.

In conclusion, among AA infants we have found a genetic polymorphism (CT and TT genotypes of the IL-4 C-589T SNP) that significantly modifies the effects of an environmental exposure (high ETS exposure) on the development of wheezing without a cold at age one. This result could be used to identify a genetically susceptible population in who increased smoking cessation efforts may significantly modify the burden of wheezing in infants.

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Appendix I. Primers and Probes Used for Genotyping of the IL-4 and IL-13 Polymorphisms

For IL-4 C-589T, the forward primer was GGCCTCACCTGATACGA, the reverse primer was TTGGAAACTGTCCTGTCAT, the sensor probe was AACATTGTCCCCCAGTGC-Fluorescein, and the anchor probe was LC Red 640-GGGTAGGAGAGTCTGCCTGTTATTCT-Phosphate

For IL-13 C-1112T, the forward primer was GGGAGAAATCTTGACATCAAC, the reverse primer was GCAGAATGAGTGCTGTG, the sensor probe was AGGAAAACGAGGGAAGAGCAG-Fluorescein, and the anchor probe was LC Red 640-AAAGGCGACATGGCTGCAG-Phosphate

Appendix II. Univariate Analysis and Multiple Logistic Regression of Wheezing Without a Cold at Age 1 in Non-AA Subgroups

	Univariate Analysis		Multiple Logistic Regression	
Factor	***P* value**	**OR (95% CI)**	***P* value**	**OR (95% CI)**
Results for non-AA infants with no history of paternal asthma (n = 494)				
Average Endotoxin Exposure (OR for 100 unit increase)	.021*	0.77 (0.61-0.96)*	.05*	0.79 (0.63-1.00)*
Cat ownership	.01*	1.86 (1.14-3.03)*	.05*	1.74 (1.003-3.01)*
Results for non-AA infants with at least 1 positive SPT (n = 153)				
Paternal asthma	.08	2.43 (0.91-6.52)	.02*	5.94 (1.35-26.06)*
Cat ownership	.02*	2.83 (1.22-6.59)*	.04*	4.08 (1.08-15.41)*
Mold + SPT	.05*	2.26 (1.01-5.08)*	.12	2.07 (0.83-5.11)

Multiple logistic regression analyses controlled for sex, pet ownership, income (<$40,000 or ≥$40,000), and proximity to traffic.
*$P \leq .05$.