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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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