I, Andrew M. Smith, hereby submit this work as part of the requirements for the degree of:

Master of Science - Molecular Epidemiology in Children’s Environmental Health

in:

Department of Environmental Health
College of Medicine

It is entitled:

Environmental Tobacco Smoke and IL-4 Polymorphism (C-589T) Gene:Environment Interaction Increases Risk of Wheezing in African-American Infants: The Cincinnati Childhood Allergy and Air Pollution Study (CCAAPS)

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Environmental Tobacco Smoke and IL-4 Polymorphism (C-589T) Gene:Environment Interaction Increases Risk of Wheezing in African-American Infants: the Cincinnati Childhood Allergy and Air Pollution Study (CCAAPS).

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by

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Abstract

Background: Children exposed to environmental tobacco smoke (ETS) have increased asthma exacerbations. IL-4 C-589T and IL-13 C-1112T single nucleotide polymorphisms are associated with enhanced production of respective cytokines and asthma phenotypes. Increased IL-4 and IL-13 cytokine levels reported in smokers suggest a potential gene-environment interaction.

Objective: The purpose of this study was to determine if infants exposed to ETS having the IL-4 or IL-13 gene polymorphisms were at increased risk of infantile wheezing.

Methods: A prospective birth cohort of 758 infants born to atopic parents 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 polymorphisms.

Results: The interaction of exposure to high levels of ETS and the CT/TT genotypes for IL-4 C-589T showed a significant association with wheezing without a cold (OR: 10.84; 95% CI: 1.12-104.64, p=0.04) in African-American infants but was not significant in non-African-Americans.
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Introduction

Genetic and environmental factors contribute to the pathogenesis of asthma. Since 1980, the prevalence, morbidity, and mortality of asthma have increased dramatically in the United States, particularly among African-Americans (AA), emphasizing the importance of environmental causations. One environmental risk factor for asthma is environmental tobacco smoke (ETS) exposure. In animal models, increased inflammation and oxidative stress as well as increased airway hyperresponsiveness have been observed with exposure to ETS. Children exposed to ETS have increased asthma exacerbations, wheezing, bronchial hyperreactivity, and impaired lung function. Further, maternal smoking during pregnancy has been associated with increased physician diagnosed asthma in children.

As for genetic factors, interleukin 4 (IL-4) and interleukin 13 (IL-13) are of particular interest in atopic disease. IL-4 influences the generation and regulation of allergic inflammation in asthma. A genetic variant of IL-4, the promoter single nucleotide polymorphism (SNP) C-589T was first identified in 1995 and was associated with increased IgE levels in asthmatic families. The C-589T has functional relevance, with increased reporter gene activity in vitro. In several populations, the C-589T variant has been associated with asthma and asthma severity. IL-13 also has prominent effects on airway hyperresponsiveness and mucus production. In asthmatic children, increased IL-13 levels have been observed in bronchial and nasopharyngeal tissue. The first polymorphism of IL-13, C-1112T (also C-1111T or C-1055T), was identified in 1999, adjacent to NF-AT site in the IL-13 promoter. The TT genotype has increased binding of nuclear proteins and decreased inhibition of IL-13 when exposed to anti-CD2 antibody. In several populations, C-1112T has been associated with asthma and serum IgE, a marker of atopic disease.
polymorphisms, however, may act in concert with ETS exposure to amplify the risk for childhood wheeze and asthma. Furthermore, the impact of the gene-environment interaction on wheezing in infancy has not been investigated.

Exposure to ETS can modulate immune responses by affecting cytokine production\textsuperscript{32-35}. Increased IL-4 levels in the lungs of mice exposed to ETS have been reported\textsuperscript{36}. In humans, IL-4 levels were found to be increased in smokers and in nasopharyngeal aspirates of those exposed to ETS\textsuperscript{34,35,37}. Increased IL-13 levels in the blood and nasopharyngeal aspirates of those exposed to ETS were reported\textsuperscript{27,34,38,39}. The increased IL-4 and IL-13 cytokine levels reported in smokers suggest a potential gene-environment interaction.

The study objective was to determine if infants who were exposed to high levels of ETS and who possessed IL-4 or IL-13 gene polymorphisms were at increased risk for the development of wheezing in the first year of life. Our hypothesis was that the genotype of the IL-4 C-589T and IL-13 C-1112T SNPs would significantly modify the effect of environmental tobacco smoke exposure on wheezing at one year of age.
Methods

Recruitment and Enrollment

The Cincinnati Childhood Allergy and Air Pollution Study (CCAAPS) is an ongoing longitudinal birth cohort study. The children were identified by birth records from October 2001 to July 2003. As previously published, addresses obtained from birth records were geocoded and distance to the nearest major highway calculated \(^{40}\). Per the initial aims of CCAAPS, children were recruited based on estimated exposure to diesel exhaust particulates, defined as exposed (<400m from a major highway) or unexposed (>1500m from a major highway).

Parents were recruited and screened for allergy symptoms \(^{41}\). To confirm their atopic status, parents who reported one or more symptoms underwent skin prick testing (SPT) with a panel of 15 common aeroallergens. Children with at least one confirmed atopic parent were enrolled to generate a cohort at high risk for development of allergic disorders. IRB approval was obtained through the University of Cincinnati.

Racial status was defined as African-American (AA) or non-African-American (non-AA). The race of the infant was determined by parental report of infant race on the questionnaire.

Data Collection

All infants were evaluated by an interviewer-administered questionnaire completed by the accompanying parent, evaluation by a health care professional, and by skin prick testing. The questionnaire was adapted from the well validated International Study of Allergies and Asthma in Children (ISAAC) \(^{42,43}\).

Infants were evaluated by SPT on the back using standardized extracts and bifurcated Accu-set\(^{\text{TM}}\) skin prick test devices (ALK-Abelló, Inc., Round Rock, TX). Subjects were skin
tested with 15 aeroallergen extracts including: pollen (timothy, meadow fescue, maple/box elder mix, white oak, red cedar, American elm, short ragweed), mold (*Alternaria alternata*, *Penicillium* mix, *Cladosporium, Aspergillus fumigatus*), pet (cat, dog), house dust mite mix (*Dermatophagoides pteronyssinus* and *D. farinae*), and German cockroach (*Blatella germanica*). Infants were also tested with commercial cow’s milk and egg white extracts (ALK-Abelló, Inc., Round Rock, TX). A positive SPT was defined as a wheal $\geq$ 3 mm larger than the saline control after 15 minutes $^{44, 45}$.

**Outcome Definitions**

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. One or more episodes of infant wheezing without a cold from the questionnaire was the outcome of interest $^{41, 46, 47}$.

**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 $^{48}$. ETS exposure was categorized as high ($\geq$ 20 cigarettes/day) or low/none (0-19 cigarettes/day).

Traffic exposure was defined as unexposed, exposure to moving traffic, or exposure to stop-and-go traffic as previously reported $^{40}$. Exposure to moving traffic was defined as living within 400m of an interstate or within 100m of a state route with a speed limit $\geq$50 miles per
hour. Exposure to stop-and-go traffic was defined as living within 100m of a bus route and/or within 100m of state route with a speed limit <50 miles per hour.

Exposure to endotoxin was determined by dust sampling and analysis as previously described. House dust samples were collected by vacuuming a 2-m² area during a home visit from the floor of the infant’s primary activity room. The concentrations of endotoxin were determined using the limulus amebocyte lysate test (Associates of Cape Cod Inc, Falmouth, Mass).

DNA

At the initial 12 month visit, buccal swab samples were collected from infants. The samples were collected by rubbing a cytology brush on the inside of the cheek for thirty seconds and then placing the brush in a centrifuge tube. Genomic DNA was extracted using the ZR Genomic DNA II Kit™ (Zymo Research Corp, Orange, CA). Briefly, the cytology brush was rinsed into a microcentrifuge tube using 500 μL of Genomic Lysis Buffer (Zymo Research Corp.). The tubes were then centrifuged, the supernatant transferred to a Zymo-Spin Column (Zymo Research Corp.), then centrifuged again. Next, 500 μL of g-DNA Wash Buffer (Zymo Research Corp.) was added and the sample centrifuged again. Finally, the DNA was eluted from the column using 40 μL of water (equilibrated to 65 °C). The DNA samples were genotyped for the IL-4 C-589T and IL-13 C-1112T polymorphisms using the Roche LightTyper platform (based on fluorescence resonance energy transfer) and specific fluorescent probes. Primers and probes were designed using Roche’s LightCycler® Probe Design Software 2.0. For IL-4 C-589T, the forward primer was GGCCTCACCTGATACGA, the reverse primer was TTGGAAACTGTCCTGTCAT, the sensor probe was AACATTGTCCCCAGTGC-Fluorescein, and the anchor probe was LC Red 640-GGGTAGGAGAGTCTGCTGTTATTCT-
Phosphate. For IL-13 C-1112T, the forward primer was GGGAGAAATCTTTGACATCAAC, the reverse primer was GCAGAATGAGTGCTGTG, the sensor probe was AGGAAAACGAGGGAAGAGCAG-Fluorescein, and the anchor probe was LC Red 640-AAAGGCGACATGGCTGCAG-Phosphate. No linkage disequilibrium between IL-4 C-589T and IL-13 C-1112T has previously been identified. A 10% random resampling was performed to assess the accuracy of the genotype data and revealed greater than 96% agreement.

Data Analysis

Given the fixed size of this birth cohort, a power calculation was performed. With a baseline rate of wheezing without a cold of 26.2%, genotype frequency of 35-40%, exposure to high ETS levels of 25%, and a genetic RR of 3, the power of the study to detect a statistically significant effect (p=0.05) was calculated to be 92%.

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) using a chi-squared analysis. If the total study population was not in HWE, the analysis was then stratified by race to minimize confounding by admixture.

Factors potentially associated with wheezing without a cold were initially evaluated by univariate logistic regression. Any factor with a p-value ≤ 0.15 by the univariate analysis was considered for inclusion in the multiple logistic regression model. Once all factors were identified for inclusion in the multiple logistic regression, “backward elimination” was performed to remove non-significant variables. The analysis was adjusted for potential factors previously reported to be associated with early wheezing including sex, pet ownership, income (<$40,000 or ≥$40,000), maternal and paternal asthma, and proximity to traffic. Unadjusted and
adjusted odds ratios (OR) and 95% confidence intervals (CI) were calculated. A p-value of < 0.05 was considered statistically significant.
Results

Demographics of the Study Population

Of the 758 infants enrolled in the CCAAPS study, 710 returned for the 12 month visit and 560 agreed to provide a DNA sample. The demographic and personal characteristics of the study population are summarized in Table I. The study population was diverse, with good regional representation of AA infants (19.4%) and lower income households (34.5%). Of the non-AA infants, 96% were Caucasian. 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 one 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 one of 17 allergens.

Exposures of the Study Population

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 population. Of the 710 infants, 300 (45.4%) reported dog ownership, and 282 (42.4%) reported cat ownership. The majority of infants (476 (69%)) in the study had been breast fed for at least one week. 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%) of the infants.

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

For the IL-4 C-589T SNP, 519 samples were available for analysis, of which 428 (82.5%) were successfully genotyped. For the IL-13 C-1112T SNP, 560 samples were available for analysis, of which 491 (87.7%) were successfully genotyped. The genotypes were evaluated
for HWE (Table II). For the total study population, the genotypes for the IL-4 SNP and IL-13 SNP were not in HWE, indicating possible population substructuring due to admixture (different disease rates and allele frequencies seen in populations of mixed ancestry). Further analyses were then stratified by race to minimize the effects of admixture.

Among AA infants, 29 (44.6%) had one mutant allele (CT) and 26 (40.0%) had two mutant alleles (TT) for IL-4 C-589T. For IL-13 C-1112T, 38 (50.0%) had one mutant allele (CT) and 13 (17.1%) were double mutants (TT). Among the AA infants, both the IL-4 C-589T and IL-13 C-1112T genotypes were in HWE (Table II).

Among non-AA infants, 78 (21.5%) had one mutant allele (CT) for IL-4 C-589T and 17 (4.7%) were double mutants (TT). For IL-13 C-1112T, 113 (27.2%) had one mutant allele (CT) and 29 (7.0%) had two mutant alleles (TT). Among the non-AA infants, neither genotype was in HWE most likely due to population substructure.

**Univariate Analysis**

Given the genetic results, the univariate analysis with the outcome of wheezing without a cold at age one was stratified by race (Table III). Among the AA infants, three factors were statistically significant (p≤0.05). Low income was associate with an increased odds of wheezing (Odds Ratio (OR) 3.49; 95% Confidence Interval (CI) 1.12-10.88; p=0.03) as was skin prick test positivity to any pollen (OR 3.75; 95% CI 1.23-11.39; p=0.02). 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 ten fold association (OR 10.00; 95% CI 1.08-92.49; p=0.04). Another approach to evaluating the data among the AA infants was to examine the proportion with wheezing without a cold based on genotype and ETS exposure (Figure 1). 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. 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 one factor was statistically significant in the
univariate analysis. History of paternal asthma was strongly associated with an increased odds
of wheezing (OR 2.21; 95% CI 1.27-3.86; p<0.01). 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=538) (Table IV). Among this subgroup, two 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=0.021), while
cat ownership was associated with an increased odds of wheezing (OR 1.86; 95% CI 1.14-3.03;
p=0.013). A further subgroup analysis was performed among the non-AA infants, evaluating
only those infants with at least one positive SPT (n=161) (Table IV). Among this subgroup, two
factors were significant in the univariate analysis. Cat ownership and SPT positive to any mold
were associated with an increased odds of wheezing (OR 2.83; 95% CI 1.22-6.59; p=0.016 | OR
2.26; 95% CI 1.01-5.08; p=0.048).

Results of the Multiple Logistic Regression

Multiple logistic regression was performed including factors identified in the univariate
analysis (Tables III and IV). The analysis was stratified by race and subgroup analysis was
performed as in the univariate analysis (Table V). 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=0.04) (Figure 2). SPT positive for any pollen was also found to be significantly associated
with wheezing (OR 6.65; 95% CI 1.15-38.44; p=0.04)
Among the non-AA infants, paternal asthma history remained the dominant effect (OR 2.13; 95% CI 1.13-4.04; p=0.02). Average endotoxin exposure, however, did not reach statistical significance for association with wheezing (OR 0.84; 95% CI 0.70-1.02; p=0.08).

In the subgroup analysis of non-AA infants without a history of paternal asthma, two 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=0.05).

In the subgroup analysis of non-AA infants with at least one positive SPT, two 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=0.02). Cat ownership was also associated with an increased odds of wheezing (OR 4.08; 95% CI 1.08-15.41; p=0.04).
Discussion

The present study is unique in examining these particular cytokine promoter variants (IL-4 C-589T and IL-13 C-1112T) - ETS exposure interaction in infants. Our study demonstrates that among AA infants, while 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 increases the odds for wheezing without a cold at age one. The same effect was not seen for IL-13 C-1112T genotype and ETS exposure.

From a public health perspective, our findings may explain the disparate burden of asthma among AA children. A first risk factor is ETS exposure. ETS exposure is very common, with an estimated smoking rate in 2004 of 20.9% of US adults. In this cohort, the smoking rate was even higher, with 33.3% of AA parents reporting current smoking and 26.9% of non-AA parents reporting current smoking. Given the increased exposure to ETS in the AA infants in this study and the known health effects of ETS exposure on early respiratory health, it is not surprising that the AA infants had an increased odds of wheezing without a cold. A second risk factor is the IL-4 C-589T genotype. The genetic differences between the two 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. The IL-4 C-589T genotype frequencies in non-AA infants in this study were similar to what has been reported in the literature. In African-Americans, the mutant allele frequencies are much higher (T: 0.54-0.67; C: 0.33-0.46) as compared to in Caucasians. 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, AA infants appear to have an increased risk at baseline.
In our study, neither ETS exposure nor IL-4 C-589T genotype alone explains the effects seen. The magnitude of the effect of the high ETS exposure x IL-4 C-589T CT/TT genotype interaction is striking (OR 10.84) and independent of other factors. This ten-fold increased odds is indicative of a synergistic effect that is greater than the additive effects of either factor alone (Figure 2). African-American children carry the mutant alleles of IL-4 C-589T at a higher rate and are exposed to ETS at a higher rate in this study, creating a highly susceptible population and leading to a disparate burden of early wheezing in these infants.

Among the non-AA infants, different effects were seen. High ETS exposure, IL-4 SNP genotype, IL-13 SNP genotype, and the gene x environment interaction were not significant. In contrast to the AA infants, only a history of paternal asthma was found to significantly increase the odds of wheezing in non-AA infants by multiple logistic regression.

In the subgroup analyses of non-AA infants, further effects were seen. Among non-AA infants with no history of paternal asthma, higher average endotoxin exposure was associated with decreased odds of wheezing. In contrast, cat ownership increased the odds of wheezing. Among non-AA infants with at least one 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 \( ^{59} \) or paternal \( ^{60} \), is a risk factor for the development of both wheezing and asthma in children \( ^{61,62} \). In this study, only paternal asthma was a significant risk factor for wheezing without a cold. Furthermore, this effect was only evident in the non-AA children. It is therefore possible that paternal asthma history may be a confounder for some other genetic polymorphism in non-AA infants.
Our results indicating an inverse association between endotoxin levels and wheezing are consistent with reports in the literature. One explanation for this effect is that high endotoxin exposure may increase the T regulatory cells of the immune system thus protecting infants at high risk of asthma against allergen sensitization and further atopic disease. While pet ownership has been shown to increase endotoxin levels and might therefore provide some protection from the development of atopic disease, 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.

In conclusion, this is the first epidemiologic study to examine this particular gene x environment (ETS) interaction in one year old infants. 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. While wheezing in the first year of life may be a poor predictor of later development of childhood asthma, as the cohort ages and asthma can be diagnosed we will be able verify and further investigate this relationship between the IL-4 C-589T SNP and ETS exposure.
Table I. Demographic and Personal Characteristics of the Study Population.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Number*</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Child’s mean age at first visit</td>
<td>13.4 ± 2.2 months</td>
<td></td>
</tr>
<tr>
<td>Infant race</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non-AA†</td>
<td>607</td>
<td>80.6</td>
</tr>
<tr>
<td>AA</td>
<td>146</td>
<td>19.4</td>
</tr>
<tr>
<td>Income &lt;$40,000</td>
<td>251</td>
<td>34.5</td>
</tr>
<tr>
<td>Male gender</td>
<td>410</td>
<td>54.6</td>
</tr>
<tr>
<td>Infant with at least one positive SPT</td>
<td>204</td>
<td>29.2</td>
</tr>
<tr>
<td>Paternal asthma</td>
<td>85</td>
<td>11.9</td>
</tr>
<tr>
<td>Maternal asthma</td>
<td>165</td>
<td>22.6</td>
</tr>
<tr>
<td>Pet ownership</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dog</td>
<td>300</td>
<td>45.4</td>
</tr>
<tr>
<td>Cat</td>
<td>282</td>
<td>42.4</td>
</tr>
<tr>
<td>None</td>
<td>248</td>
<td>38.6</td>
</tr>
<tr>
<td>Breast fed at least one week</td>
<td>476</td>
<td>68.8</td>
</tr>
<tr>
<td>High ETS exposure (≥ 20 cigarettes/day)</td>
<td>100</td>
<td>13.7</td>
</tr>
<tr>
<td>Traffic exposure</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Moving</td>
<td>167</td>
<td>27.6%</td>
</tr>
<tr>
<td>Stop-and-go</td>
<td>89</td>
<td>14.7%</td>
</tr>
</tbody>
</table>

*Number of infants may vary due to missing information
†Of the non-AA infants, 96% were Caucasian.

Abbreviations: AA=African-American, SPT=skin prick test, ETS=environmental tobacco smoke
Table II. Genotype Frequencies of the IL-4 C-589T and IL-13 C-1112T SNPs by Racial Categories.

<table>
<thead>
<tr>
<th>Race</th>
<th>Genotype</th>
<th>IL-4 C-589T SNP</th>
<th>IL-13 C-1112T SNP</th>
</tr>
</thead>
<tbody>
<tr>
<td>All</td>
<td>CC</td>
<td>278 (65.0%)</td>
<td>298 (60.7%)</td>
</tr>
<tr>
<td></td>
<td>CT</td>
<td>107 (25.0%)</td>
<td>151 (30.8%)</td>
</tr>
<tr>
<td></td>
<td>TT</td>
<td>43 (10.1%)</td>
<td>42 (8.5%)</td>
</tr>
<tr>
<td></td>
<td>HWE</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>Non-AA</td>
<td>CC</td>
<td>268 (73.8%)</td>
<td>273 (65.8%)</td>
</tr>
<tr>
<td></td>
<td>CT</td>
<td>78 (21.5%)</td>
<td>113 (27.2%)</td>
</tr>
<tr>
<td></td>
<td>TT</td>
<td>17 (4.7%)</td>
<td>29 (7.0%)</td>
</tr>
<tr>
<td></td>
<td>HWE</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>AA</td>
<td>CC</td>
<td>10 (15.4%)</td>
<td>25 (32.9%)</td>
</tr>
<tr>
<td></td>
<td>CT</td>
<td>29 (44.6%)</td>
<td>38 (50.0%)</td>
</tr>
<tr>
<td></td>
<td>TT</td>
<td>26 (40.0%)</td>
<td>13 (17.1%)</td>
</tr>
<tr>
<td></td>
<td>HWE</td>
<td>Yes</td>
<td>Yes</td>
</tr>
</tbody>
</table>

Abbreviations: SNP=single nucleotide polymorphism, AA=African-American, HWE=Hardy-Weinberg equilibrium
Table III. Univariate Analysis of Wheezing without a Cold at Age One by Race.

<table>
<thead>
<tr>
<th>Factor</th>
<th>AA Infants*</th>
<th>Non-AA Infants†</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>P Value</td>
<td>OR (95% CI)</td>
</tr>
<tr>
<td>Low Income (&lt;$40,000)</td>
<td>0.03</td>
<td>3.49 (1.12-10.88)</td>
</tr>
<tr>
<td>Pollen + SPT</td>
<td>0.02</td>
<td>3.75 (1.23-11.39)</td>
</tr>
<tr>
<td>Paternal asthma</td>
<td> </td>
<td> </td>
</tr>
<tr>
<td>IL-4 C-589T (CT/TT) x High ETS exposure</td>
<td>0.04</td>
<td>10.00 (1.08-92.49)</td>
</tr>
</tbody>
</table>

Bold = p ≤ 0.05

Abbreviations: AA=African-American, SPT=skin prick test, ETS=environmental tobacco smoke

*AA Infants
Considered for inclusion in multiple logistic regression based on p ≤ 0.15: Moving traffic exposure, SPT + to any food
P > 0.15: Male gender, breast fed ≥1 week, high ETS exposure, stop-and-go traffic exposure, average endotoxin exposure, cat ownership, dog ownership, SPT + to mold, milk, egg, dust mite, cockroach, cat, dog, maternal asthma, paternal asthma, IL-4 C-589T genotype, IL-13 C-1112T genotype, IL-13 C-1112T x high ETS interaction, moving or stop-and-go traffic exposure x IL-4 or IL-13 genotype interaction

†Non-AA Infants
Considered for inclusion in multiple logistic regression based on p ≤ 0.15: Male gender, low income (<$40,000), average endotoxin exposure, cat ownership, SPT + to mold
P > 0.15: Breast fed ≥1 week, high ETS exposure, stop-and-go traffic exposure, moving traffic exposure, dog ownership, SPT + to pollen, milk, egg, dust mite, cockroach, cat, dog, maternal asthma, IL-4 C-589T genotype, IL-13 C-1112T genotype, IL-4 or IL-13 genotype x high ETS interaction, moving or stop-and-go traffic exposure x IL-4 or IL-13 genotype interaction
Table IV. Subgroup Analysis of Non-AA Children with no History of Paternal Asthma or with at Least One Positive SPT.

<table>
<thead>
<tr>
<th>Factor</th>
<th>Non-AA Infants with No History of Paternal Asthma*</th>
<th>Non-AA Infants with at Least One Positive SPT†</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>P Value</td>
<td>OR (95% CI)</td>
</tr>
<tr>
<td>Average Endotoxin Exposure (OR for 100 unit increase)</td>
<td><strong>0.021</strong> *</td>
<td><strong>0.77 (0.61-0.96)</strong></td>
</tr>
<tr>
<td>Cat ownership</td>
<td><strong>0.01</strong></td>
<td><strong>1.86 (1.14-3.03)</strong></td>
</tr>
<tr>
<td>Mold + SPT</td>
<td>0.05</td>
<td><strong>2.26 (1.01-5.08)</strong></td>
</tr>
</tbody>
</table>

**Bold** = p≤0.05

Abbreviations: AA=African-American, SPT=skin prick test

*Non-AA Infants with No History of Paternal Asthma
  Considered for inclusion in multiple logistic regression based on p≤0.15: Male gender, low income (<$40,000), SPT + to mold, any pet
  P>0.15: Breast fed for at least one week, high ETS exposure, stop-and-go traffic exposure, moving traffic exposure, dog ownership, SPT + to pollen, milk, egg dust mite, cockroach, cat, dog, maternal asthma, IL-4 C-589T genotype, IL-13 C-1112T genotype, IL-4 or IL-13 genotype x high ETS interaction

†Non-AA Infants with at Least One Positive SPT†
  Considered for inclusion in multiple logistic regression based on p≤0.15: Breast fed for at least one week, high ETS exposure, dog ownership, IL-4 C-589T x high ETS interaction
  P>0.15: Male gender, low income (<$40,000), stop-and-go traffic exposure, moving traffic exposure, average endotoxin exposure, SPT + to pollen, milk, egg, dust mite, cockroach, cat, dog, maternal asthma, IL-4 C-589T genotype, IL-13 C-1112T genotype, IL-13 C-1112T x high ETS interaction, moving or stop-and-go traffic exposure x IL-4 or IL-13 genotype interaction
Table V. Multiple Logistic Regression with the Outcome of Wheezing without a Cold at Age One by Race and Subgroups.

**AA**

<table>
<thead>
<tr>
<th>Factor</th>
<th>OR</th>
<th>95% CI</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pollen + SPT</td>
<td><strong>6.65</strong></td>
<td><strong>1.15-38.44</strong></td>
<td><strong>0.04</strong></td>
</tr>
<tr>
<td>High ETS Exposure and IL-4 C-589T CT/TT</td>
<td><strong>10.84</strong></td>
<td><strong>1.12-104.64</strong></td>
<td><strong>0.04</strong></td>
</tr>
</tbody>
</table>

**Non-AA**

<table>
<thead>
<tr>
<th>Factor</th>
<th>OR</th>
<th>95% CI</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Average Endotoxin Exposure (OR for 100 unit increase)</td>
<td>0.84</td>
<td>0.70-1.02</td>
<td>0.08</td>
</tr>
<tr>
<td>Paternal asthma</td>
<td><strong>2.13</strong></td>
<td><strong>1.13-4.04</strong></td>
<td><strong>0.02</strong></td>
</tr>
</tbody>
</table>

**Non-AA, no paternal asthma**

<table>
<thead>
<tr>
<th>Factor</th>
<th>OR</th>
<th>95% CI</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Average Endotoxin Exposure (OR for 100 unit increase)</td>
<td><strong>0.79</strong></td>
<td><strong>0.63-1.00</strong></td>
<td><strong>0.05</strong></td>
</tr>
<tr>
<td>Cat ownership</td>
<td><strong>1.74</strong></td>
<td><strong>1.003-3.01</strong></td>
<td><strong>0.05</strong></td>
</tr>
</tbody>
</table>

**Non-AA, at least 1 positive SPT**

<table>
<thead>
<tr>
<th>Factor</th>
<th>OR</th>
<th>95% CI</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cat ownership</td>
<td><strong>4.08</strong></td>
<td><strong>1.08-15.41</strong></td>
<td><strong>0.04</strong></td>
</tr>
<tr>
<td>Paternal asthma</td>
<td><strong>5.94</strong></td>
<td><strong>1.35-26.06</strong></td>
<td><strong>0.02</strong></td>
</tr>
</tbody>
</table>

**Bold** = p≤0.05

Abbreviations: AA=African-American, SPT=skin prick test, ETS=environmental tobacco smoke
Figures

Figure 1. Wheezing without a Cold at Age One in African-American Infants by IL-4 C-589T Genotype and Environmental Tobacco Smoke (ETS) Exposure.
Figure 2. Interaction of IL-4 C-589T Genotype and Environmental Tobacco Smoke (ETS) Exposure in AA Infants.

For each factor, numbers are odds ratio, 95% confidence interval, and p-value.
* IL-4 C-589T and high ETS exposure results from univariate analysis, interaction results from multiple logistic regression
References


