Alternaria is associated with asthma symptoms and exhaled NO among NYC children

To the Editor:

The importance of domestic *Alternaria* exposure to allergic disease in urban communities is underrecognized relative to cockroach, dust mite, and mouse exposures.[1,2] Concentrations of *Alternaria* are higher in outdoor than in indoor air; however, dampness, leaks, and resident behaviors can influence fungal penetrance and secondary growth indoors.[3] Moreover, given the amount of time children spend indoors, domestic exposure may contribute more than outdoor exposure to asthma morbidity.

Byproducts from fossil fuel combustion are common in urban air and are the most well-established anthropogenic environmental adjuvants of allergic sensitization.[4] Black carbon and elemental carbon (EC), indicators of combustion exposure, vary across cities such as New York (NYC) because of vehicle and residential heating sources.[5,6] Among NYC children, we previously demonstrated an interaction between exposure to combustion byproducts and cockroach allergen on cockroach sensitization[7] and an association between black carbon measured inside homes and fractional exhaled nitric oxide (FENO), a marker of airway inflammation.[5] Therefore, combustion byproducts might enhance the effect of fungal exposure on allergic disease outcomes among NYC children.

We hypothesized that, among NYC children, (1) sensitization to *Alternaria alternata* would be associated with increased asthma symptoms, (2) domestic exposure to *A alternata* would be associated with increased FENO, and (3) the association with FENO would be modified by neighborhood EC concentrations.

The NYC Neighborhood Asthma and Allergy Study is an asthma case-control study. Seven- to eight-year-old children were recruited through a health insurance provider primarily serving middle-income families.[5] Study details and demographic characteristics (see Table E1 in this article's Online Repository at www.jacionline.org) for children included (n = 270) are available in this article's Online Repository at www.jacionline.org. FENO and serum IgE to inhalant allergens, including *A alternata* and mixed fungal species (Mx2, Phadia, Uppsala, Sweden), were measured.[5] *A alternata* was measured by quantitative PCR from sieved settled dust samples collected from the child's bedroom floor.[8] Children's neighborhood annual airborne EC was estimated using published data.[6]

A alternata was detected in 85% of homes, ranging from less than 10 to 33,158 spore equivalents/mg dust collected (geometric mean = 57 spores equivalents/mg), which was approximately 60% higher than was measured in a study of US homes.[8] In bivariate analyses, *A alternata* concentrations were associated with neighborhood asthma prevalence, housing type, carpeting, and household and neighborhood income (all $P < .001$; see Tables E2 and E3 in this article's Online Repository at www.jacionline.org). Higher *A alternata* concentrations were observed with wet mopping ($P = .020$) and inversely correlated with neighborhood EC ($P = .029$). In multivariable analyses, only the presence of carpet remained independently associated with *A alternata* ($P = .003$; see Table E4 in this article's Online Repository at www.jacionline.org).

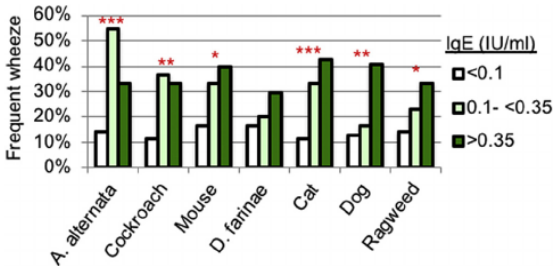

FIG 1. Prevalence of frequent wheeze symptoms among children with asthma by IgE response to *A alternata* and other inhalant allergens (n = 157). Asterisks denote statistical differences in frequency between IgE groups. *$P < .05$, **$P < .01$, ***$P < .001$.

IgE to *Alternaria* was detected in 6.3% of children (n = 269) using the standard cutoff point of greater than or equal to 0.35 international units (IU)/mL. However, an additional 10% of children had IgE detectible in the greater than or equal to 0.1 to less than 0.35 IU/mL range (ie, 16% had IgE ≥0.1 IU/mL). Sensitization at both lower and higher cutoff points was more common among asthma cases than among nonasthmatic controls ($P = .016$) and was associated with a similar likelihood of having detectible IgE 3 years later (see this article's Online Repository at www.jacionline.org). Among asthma cases, children sensitized to *A alternata* were more likely than nonsensitized children to report frequent wheeze (≥4 episodes) in the past year (Fig 1), including those with sensitization in the greater than or equal to 0.1 to less than 0.35 IU/mL range. These findings support lowering the commonly used IgE cutoff point of 0.35 to 0.1 IU/mL to identify children potentially susceptible to greater asthma morbidity with *A alternata* sensitization for epidemiological studies.

In multivariable models controlling for sex, African American race, Hispanic ethnicity, maternal asthma, smoker in the home, and season, sensitization to *A alternata* (≥0.1 IU/mL) was associated with frequent wheeze (prevalence ratio = 3.7; 95% CI, 1.8-7.8; $P < .001$) and other asthma outcomes (see Table E5 in this article's Online Repository at www.jacionline.org). This prevalence ratio remained significant in models that also adjusted for sensitization to other common inhalant allergens (eg, other fungi, cockroach, mouse, dust mite, and cat; see Fig E1 in this article's Online Repository at www.jacionline.org). These results suggest that *A alternata* may be a relevant contributor to asthma morbidity in this urban community, like cockroach, mouse, and dust mite.

A alternata in house dust was not associated with sensitization to *A alternata*, frequent wheeze, or lung function (see this article's Online Repository and Table E6 in this article's Online Repository at www.jacionline.org). However, in multivariable models, *A alternata* in dust was marginally associated with FENO (= .043; $P = .078$). Unexpectedly, this association appeared to be stronger among the children not sensitized to *A alternata* (see the Results section in this article's Online Repository at www.jacionline.org). In stratified analysis, no association was observed among the children living in lower EC neighborhoods (= −0.021; $P = .59$), but a relatively weak, but statistically significant positive association was observed among children in higher EC neighborhoods (= 0.089; $P = .008$; $P_{interaction} = .028$;

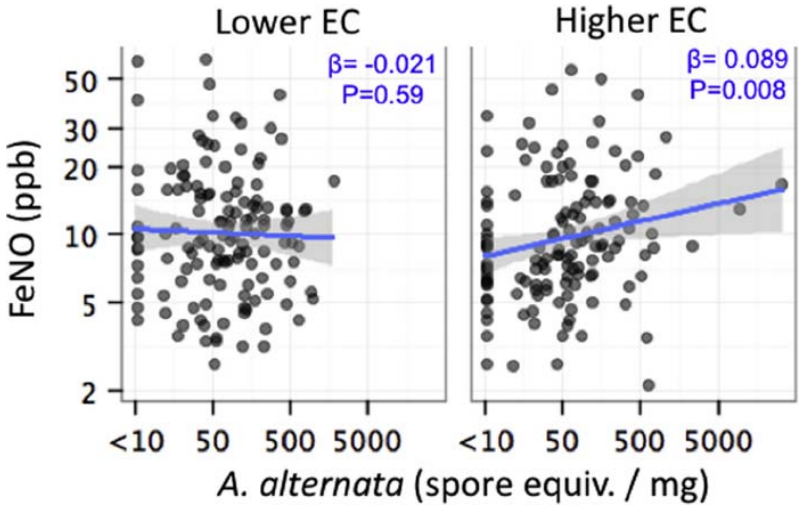

FIG 2. Correlation between *A alternata* abundance and FENO, stratified by outdoor airborne EC in child's neighborhood. Beta and *P* values are derived from liner regression models adjusted for ambient NO, sex, maternal asthma, smoker in home, black race, Hispanic ethnicity, season, seroatopy, and dust mite allergen. The beta value was statistically significantly greater among children in higher versus lower EC neighborhoods ($P_{interaction}$ = .028).

Fig 2). We also observed a similar, independent, interaction with EC on the association between cockroach allergen and FENO, but not on associations with other allergens (see the Results section and Table E7 in this article's Online Repository at www.jacionline.org). This study further implicates combustion byproducts as relevant to the allergic pathway in urban communities, lending support to large-scale public health interventions, like NYC's Clean Heat Initiative.

Cross-sectional design and a focus on 1 fungal species were limitations of this study. Other mold taxa that we did not measure may also affect the risk of allergic sensitization to *A alternata* and asthma morbidity, especially because homologs of the major allergen from *A alternata*, Alt a 1, are also produced by other species in the *Pleosporaceae* family. Thus, our species-specific nucleic acid–based measurement of *A alternata* has implications for exposure misclassification.[9]

In summary, *A alternata* appears to be relevant to asthma morbidity for children living in NYC. Using quantitative PCR, we detected *A alternata* in settled dust in most NYC homes, suggesting that domestic exposure is common. Sensitization to *A alternata*, including at concentrations of IgE previously considered to be negligible (0.1-<0.35 IU/mL), was associated with asthma morbidity, independent of sensitization to other common inhalant allergens. *A alternata* measured in settled dust was associated with increased FENO, especially among children living in neighborhoods with higher EC exposure, indicating that exposure to combustion byproducts may heighten the effect of *A alternata* exposure on urban asthma morbidity. The relatively modest associations and the observed association between exposure and FENO among the nonsensitized children call for both caution in drawing conclusions and future studies to clarify the role of *A alternata* in urban asthma morbidity.

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Evaluation of food allergy candidate loci in the Genetics of Food Allergy study

To the Editor:

Food allergy (FA) is a common health problem with a strong genetic etiology. Twin studies estimated FA heritability at about 80%,[1,2] but our knowledge of the genes underlying FA is still sparse. A recent genome-wide association study (GWAS) on FA published in the *Journal of Allergy and Clinical Immunology* investigated 850 cases from the Canadian Peanut Allergy Registry (CanPAR) and 926 Australian controls.[3] The CanPAR study successfully identified a new FA locus on chromosome 11q13, near the genes *C11orf30* (chromosome 11 open reading frame 30) and *LRRC32* (leucine-rich repeat-containing 32), and proposed additional risk variants for further investigation that did not meet the threshold of genome-wide significance.[3]

Here, we aimed to evaluate the findings of the CanPAR study in an independent study population, the German Genetics of Food Allergy (GOFA) study. The GOFA study consists of 902 cases with FA, including 342 with peanut allergy, and 3668 control individuals of unknown phenotype from 2 German population-based studies, the Heinz Nixdorf Recall Study (n = 2682) and the Study of Health in Pomerania (n = 986). A GWAS on the GOFA study has recently been published and identified 5 genome-wide significant susceptibility loci for FA and peanut allergy.[4] Most GOFA study cases were diagnosed by double-blind, placebo-controlled food challenges (Table I), the current gold standard for the diagnosis of FA.[5,6] Detailed information on clinical phenotypes, study population, genotyping, and statistical analyses is provided in this article's Methods section in the Online Repository at www.jacionline.org. After quality control, 866 cases including 336 peanut-allergic children and 3358 controls were included in our case-control association study on FA.

TABLE I. Characterization of the GOFA study

Characteristic	GOFA study
Total number of samples	902
Sex	64% males
Age (y), mean ± SD*	4.8 ± 3.6
Diagnosis, n (%)	
Double-blind, placebo-controlled food challenge	650 (72)
Oral food challenge	125 (14)
Severe allergic reaction plus elevated allergen-specific serum IgE (>0.35 kU/L)	127 (14)
Food allergies, n (%)	
Hen's egg	504 (56)
Peanut	352 (39)
Cow's milk	276 (31)

*Mean age at last visit.

In line with the CanPAR study,[3] we have previously reported genome-wide significant association of the *C11orf30/LRRC32* locus with FA.[4] Analyzing the CanPAR lead single nucleotide polymorphism (SNP), rs7936434, in the GOFA study revealed association with FA ($P = 1.6 \times 10^{-7}$) and with peanut allergy ($P = .0024$; Table II), which was significant after correction for the number of markers tested. Since rs7936434 is in high linkage disequilibrium (LD) with the previously reported, best-associated SNP of the GOFA study, rs2212434 ($r^2 = 0.89$), both variants represent the same locus. In addition, we investigated the other 7 independent SNPs, rs115218289, rs72827854, rs144897250, rs7475217, rs744597, rs523865, and rs78048444, which were suggestive of association with FA in the CanPAR GWAS. Two additional variants reported in that study (rs56151068 and rs139462954) were in very high LD with the lead variant rs72827854 ($r^2 > 0.92$) on chromosome 17, thus representing the same association. All candidate SNPs were either genotyped or imputed with high quality ($r^2 > 0.75$) in the GOFA study. None of the 7 candidate SNPs was associated with FA in our study (Table II). Because all CanPAR cases were recruited through peanut allergy, we then tested whether the reported associations were peanut-specific. However, restricting the analysis to the subset of peanut-allergic children from the GOFA study did not change the results (Table II). We used the Genetic Power calculator[7] to assess the power of our study sample for replication. Based on the allele frequencies and the reported effect sizes, the GOFA study provided nearly 100% power to detect association of the candidate variants with FA at the Bonferroni-corrected significance threshold of $P < .00714$. The power to detect a peanut-specific effect was reduced for only 2 low-frequency variants (rs115218289 and rs78048444) but still exceeded 80% for a nominal significance level (see Table E1 in this article's Online Repository at www.jacionline.org).

In the CanPAR study, 7 loci suggestive for association with FA were reported, which were not confirmed in independent study populations included in the original report.[3] Because