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).

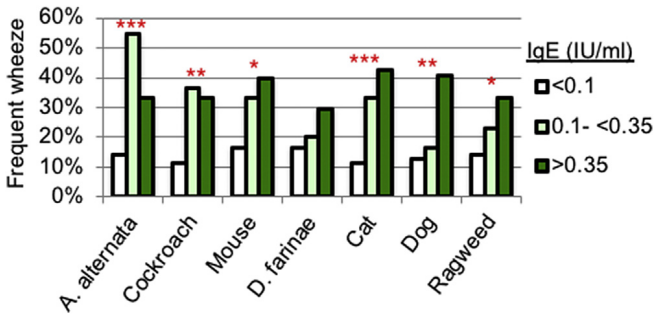

FIG 1. Prevalence of frequent wheeze symptoms among children with asthma by IgE response to *A alternata* and other inhalant allergens (n = 157). Asterisks denote statistical differences in frequency between IgE groups. *$P < .05$, **$P < .01$, ***$P < .001$.

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 ($\beta = .043$; $P = .078$). 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 ($\beta = -0.021$; $P = .59$), but a relatively weak, but statistically significant positive association was observed among children in higher EC neighborhoods ($\beta = 0.089$; $P = .008$; $P_{interaction} = .028$;

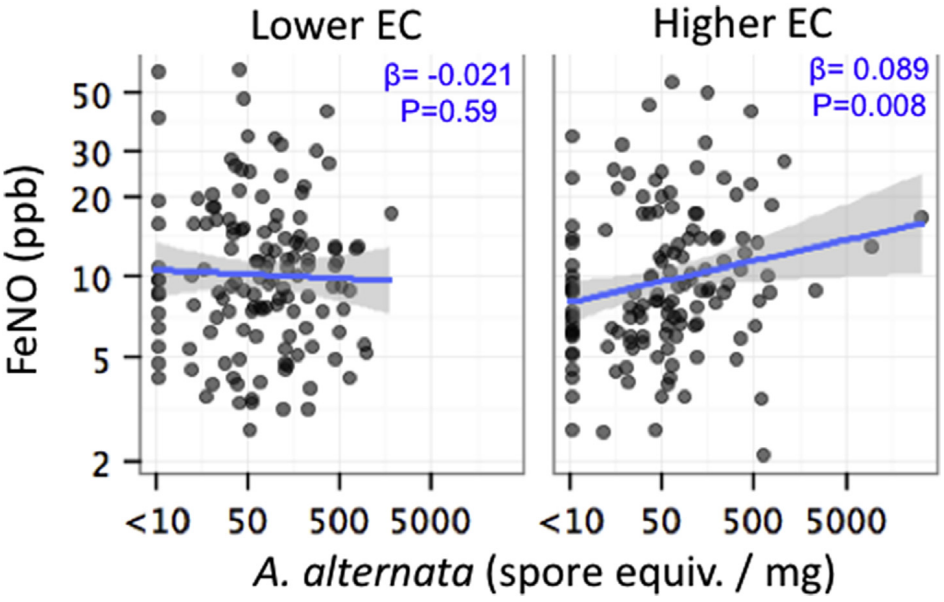

FIG 2. Correlation between *A alternata* abundance and FENO, stratified by outdoor airborne EC in child's neighborhood. Beta and *P* values are derived from liner regression models adjusted for ambient NO, sex, maternal asthma, smoker in home, black race, Hispanic ethnicity, season, seroatopy, and dust 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.

We thank the participating mothers and children. This work would not have been possible without the hard work and dedication of the research workers and field technicians. The findings and the conclusions in this report are those of the authors and do not necessarily represent the views of the National Institute for Occupational Safety and Health.

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This study was supported by the Department of Housing and Urban Development Healthy Homes Technical Studies (grant nos. NYHHU0003-11 and NYHHU0021-13), the Centers for Disease Control and Prevention (grant no. CDC–TP–13-001), and the National Institute for Environmental Health Sciences (NIEHS) (grant nos. R01 ES014400 and P30 ES009089). This study was supported by internal NIOSH funds and in part by an interagency agreement between the National Institute of

Occupational Safety and Health and NIEHS (AES12007001-1-0-6) as a collaborative National Toxicology Program research activity.

Disclosure of potential conflict of interest: The authors declare that they have no relevant conflicts of interest.

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Available online June 28, 2018.
https://doi.org/10.1016/j.jaci.2018.04.043

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

METHODS

The NYC Neighborhood Allergy and Asthma (NAAS) is a case-control study of asthma for which 7- to 8-year-old children were recruited through a health care provider used primarily by a middle-income population.[E1] Recruitment targeted children living in higher asthma prevalence neighborhoods (>11.0%) and lower asthma prevalence neighborhoods (<9.0%) in the Bronx, Queens, Brooklyn, and Manhattan. Children with complete data on *A alternata* exposure and a valid FENO measure were included in these analysis (n = 270). The children included in these analyses were not statistically significantly different in demographic characteristics from children in NYC NAAS who were not included in the analyses (n = 77; see Table E1). This study was approved by Columbia University's Institutional Review Board.

Alternaria bedroom floor dust

During the home visit, a dust sample was collected from the child's bedroom floor by vacuuming approximately 2 m^2 surrounding the bed for 3 minutes using a vacuum cleaner and Dustream collector (Indoor Biotechnologies, Charlottesville, Va). Dust was stored at −20°C until total DNA was extracted.

Dust samples were extracted and analyzed at Assured Biolabs (Oak Ridge, Tenn). Briefly, dust was sieved (300 μm), and 5 mg of dust from each sample was then extracted using a modified High Pure PCR Template Kit (Roche, Basal, Switzerland) protocol.[E2] Extracted total DNA was amplified and quantified using the Mold-Specific Quantitative Polymerase Chain Reaction (MSqPCR) technique.[E3] MSqPCR is based on 36 species-specific primers developed by the US-EPA. This was performed on an Eppendorf 5057 liquid handling device and LightCylcer 480. Species-specific quantitative DNA was used to estimate fungal spores/mg dust. Assured Bio Labs, LLC is trained and certified to perform this technique and operates under EPA license number 416-07. Although 36 species were quantified with this method, the analysis was focused on the *A alternata* abundances for this article.

Inhalant allergens in bed dust allergens

Bed dust samples were collected during home visits by vacuuming the upper half of the fitted sheet and both sides of the pillows from the child's bed using a Duststream collector for 3 minutes as described previously.[E4] Bed dust samples were extracted using PBS 0.05% Tween, pH 7.4, at a concentration of 50 mg/mL and store at −20°C until analysis. Der f 1 Fel d 1, Can f 1, and Mus m 1 were measured using multiplex bead immunoassays. Bla g2 was measured by ELISA (Indoor Biotechnologies). All results are based on the universal allergen standard curve. For results below the limit of detection (LOD), we used values of ½ the LOD. LODs and coefficients of variance for duplicates are described in a previous publication.[E4]

Relative humidity

HOBO H08-003-02 data loggers (Onset Computer Corporation, Cape Cod, Mass) were installed for periods of 6 to 13 days (average, 9.1 days; SD, 2.1 days) in the homes of the study participants, 1.5 m above the floor and away from windows and drafts, as described previously.[E5] Data logger locations were preferentially placed in the living room. These data loggers record both temperature and relative humidity, with accuracy of ±0.7°C for temperature and of ±5% for relative humidity. Measurements were recorded at 5-minute intervals, from which we calculated hourly averages, which were used to calculate an average for the sampling period.

Neighborhood airborne outdoor EC annual averages

In partnership with Queens College, NYC Department of Health conducted the NYC Community Air Survey. During 2008 and 2009, 150 outdoor air-monitoring stations were placed throughout NYC to measure, among other pollutants, EC. United Hospital Fund level (UHF level = several ZIP codes) annual average EC concentrations from this data were made publicly available by the NYC Department of Health and Mental Hygiene.[E6] These average concentrations were linked to study subject's home address by ZIP code.

Asthma case status

During a home visit, parents answered a detailed questionnaire pertaining to the child's health, demographic characteristics, and environmental exposures. Asthma cases were defined by questionnaire, including the modules from the International Study of Asthma and Allergy in Childhood as described previously.[E4,E7] Children were classified as asthmatic if their caregiver reported at least 1 of the following for the child in the past 12 months: (1) wheeze, (2) being awakened at night by cough without having a cold, (3) wheeze with exercise, or (4) report of medication use for asthma. Children not meeting the asthma definition were classified as nonasthmatic.

FENO

FENO and ambient NO measurements were collected by the offline method described previously.[E1,E8] Breath samples were considered valid only if (1) the child exhaled at a consistent, correct flow AND (2) EITHER the child inhaled correctly through the NO sampling device filter OR the ambient NO was less than 20 ppb. Samples collected when the ambient NO was more than 100 ppb were excluded regardless of proper inhalation.[E1]

IgE antibodies

IgE antibodies were measured by ImmunoCAP (Phadia, Upsalla, Sweden) in serum collected from during the home visit as previously described.[E9,E10] Antibodies were measured to common inhalant allergens, *A alternata* (M6), and a mixture of 6 fungi (MX2). The MX2 mix included antigens from *Penicillium chrysogenum*, *Cladosporium herbarum*, *Aspergillus fumigatus*, *Candida albicans*, *A alternata*, and *Setomelanomma rostrate*. Specific IgE concentrations to the specific allergens were measured using the 0.1 IU/mL lower LOD calculated by the ImmunoCAP 100. Results for the MX2 CAP are provided only as positive or negative at the 0.35 IU/mL cutoff point. Raw concentration values were used to calculate concentrations of IgE to the MX2 CAP between 0.1 and 0.35 IU/mL.

Statistical methods

Alternaria and allergen concentrations in dust were log transformed for all statistical tests, with geometric mean concentrations reported. Prevalence ratios for risk of frequent wheeze symptoms with sensitization to *Alternaria* and other allergens were calculated using a generalized estimating equation model with UHF used as a cluster variable because the EC data were available at the UHF level. Variables conventionally considered as potential confounders were included in the multivariable models.[E11] Linear regression models were tested with logarithmically transformed FENO as the dependent variable. Similar potential confounders were included in the models, along with ambient NO in the home during collection. Data were analyzed in SPSS version 24 (Chicago, Ill) and visualized in R version 3.3.1.

RESULTS

For this article, we analyzed cross-sectional associations at ages 7 to 8 years. In a subset of the children, we had another IgE measurement taken 3 years after the baseline measurement (ages 10-11 years). Of these children, 19 had IgE to *A alternata* at age 7 to 8 years in the 0.1 to less than 0.35 IU/mL range and 11 had IgE level of more than 0.35 I.U/mL at baseline. At ages 10 to 11 years, 14 of the 19 kids with lower baseline IgE (74%) and 9 of the 11 kids with higher baseline IgE levels (82%) had detectible (≥0.1 IU/mL) IgE.

There was no association between *A alternata* in house dust and sensitization to *A alternata*. The geometric mean concentrations of *A alternata* in house dust did not differ between *A alternata* sensitized and nonsensitized children ($P = .95$). The prevalence of sensitization to *A alternata* was not statistically significantly different between children with and without detectible *A alternata* in house dust (17% vs 10%; $P = .24$). Similarly, investigations in potential nonlinear associations between *A alternata* in house dust and sensitization did not reveal any associations.

When we stratified the multivariable analyses testing the association between *A alternata* and FENO by IgE to *A alternata*, the association was of borderline statistical significance in the children without IgE to *A alternata* (n = 224; $B = 0.047$; $P = .058$), but was not statistically significant among the children with IgE (n = 43; $B = -0.056$; $P = .53$), nor was the difference between these associations ($P_{\text{interaction}} = 0.30$). Given the small sample size of children with IgE, we must be cautious in our interpretation. However, considering the fact that we observed an association in the relatively large group of children without IgE, the association may be nonallergic.

Six samples had more than 1000 spore equivalents/mg of *A alternata*. We conducted sensitivity analyses of the association between *A alternata* and FENO excluding those 6 samples in models adjusted for sex, race, Hispanic ethnicity, season, neighborhood asthma prevalence, and dust mite allergen and ambient NO. Among the children in the lower EC neighborhoods, the association between *A alternata* and FENO was $\beta = -0.026$, $P = .50$ (n = 132). Among the children in the higher EC neighborhood, the association was $\beta = 0.079$, $P = .045$ (n = 131). The interaction P value was 0.051. Therefore, those high values appear to have contributed to the association, but excluding them did not eliminate the association between *A alternata* and FENO among children in the higher EC neighborhoods. We did not remove values from our main analysis because we had no reason to suspect that they are erroneous outliers; rather they may represent high concentrations that contribute to increased FENO.

To test for the independence of the relationships between *A alternata* and Bla g 2 with FENO, models were run stratifying by higher and lower EC with both *A alternata* and Bla g 2 in the same models predicting FENO (and adjusting for seroatopy, environmental tobacco smoke, asthma, ambient NO, sex, race, Hispanic ethnicity, season, neighborhood asthma prevalence). Among children in the lower EC neighborhoods, neither *A alternata* ($B = -0.023$; $P = .55$) nor Bla g 2 ($B = -0.099$; $P = .11$) was associated with FENO. Among the children in the higher EC neighborhoods, both *A alternata* ($B = 0.093$; $P = .004$) and Bla g 2 ($B = 0.085$; $P = .036$) were independently associated with FENO. The interaction between *A alternata* and neighbohorhood EC remained statistically significant ($P = .045$) after adjustment for cockroach allergen, as did the interaction between Bla g 2 and neighborhood EC ($P = .044$) after adjustment for *A alternata*.

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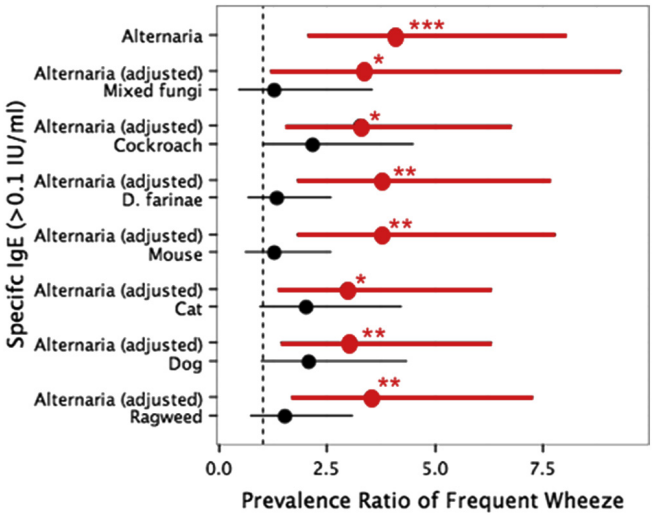

FIG E1. Association between sensitization (≥0.1 IU/mL) to *A alternata* and frequent wheeze symptoms among children with asthma, adjusting for sensitization to other inhalant allergens. Dots represent prevalence ratios (PRs) and lines represent the 95% CIs of those PR, with those for *A alternata* in red and the other allergens in black. Risks of frequent wheeze symptoms with sensitization to *Alternaria* and other allergens were calculated using a generalized estimating equation model with UHF used as a cluster variable. Models included sex, black race, Hispanic ethnicity, maternal asthma, smoker in the home, season, and IgE to other allergens (tested individually with Alternaria and the other covariates). A similar PR was observed for sensitization in the ≥0.1-<0.35 range compared with children with <0.1 IU/mL (PR = 4.3; $P = .001$). *$P < .05$, **$P < .01$, ***$P < .001$.

TABLE E1. Demographic characteristics of cohort for children included and not included in the analyses

	Included in analyses (n = 270)	Not included in the analyses (n = 77)	*P* value for difference
High asthma prevalence neighborhood, %*	50.0	49.4	.92
Asthma cases, %	58.5	59.7	.85
Sex: male, %	45.2	41.6	.57
Race, %†			
Black	48.5	40.3	.20
White	14.8	14.3	.91
Asian	10.4	14.3	.34
Other/mixed	22.2	28.6	.25
Hispanic ethnicity, %‡	29.9	35.9	.32
Household income (<$25K), %	9.3	10.4	.77
Household incomes for surrounding 500 m, median§	$34.2K	$31.5K	.57
Maternal asthma, %‖	20.8	17.3	.51
Live in apartment, %	54.8	51.9	.66
Carpeted bedroom floor, %	45.9	55.8	.12
Report of mold odor, %	12.2	6.5	.24
Report of visible mold, %	38.7	35.5	.62
Report wet mopping, %	20.7	19.7	.85
Geometric mean *A alternata* (spore equivalent/mg)	57.3	52.0	.63
Median neighborhood EC (ng/m^3)	1.1	1.1	.99

*Children not in the higher asthma prevalence neighborhoods (11.0%-19% prevalence) were in the lower asthma prevalence neighborhoods (3%-9.0% prevalence).
†There were 11 (4.1%) and 2 (2.6%) children who did not have a race reported among children included and not included in the analysis, respectively.
‡There were 1 (0.4%) and 2 (2.7%) children who did not have a report for Hispanic ethnicity among children included and not included in the analysis, respectively.
§GIS census-based variable of the median income of the household in the surrounding radian 500 m.
‖There were 5 (1.9%) and 2 (2.6%) children without a report for maternal asthma among children included and not included in the analysis, respectively.

TABLE E2. Geometric mean of *A alternata* in bedroom floor dust by home and neighborhood characteristics and household behavior

Characteristic	n	Geometric mean (95% CI) spore equivalent/mg	*P* value for difference*
Neighborhood asthma prevalence			
HAPN	135	81 (63-104)	<.001
LAPN	135	40 (31-52)	
Housing type			
Single	52	84 (58-122)	.003
Multifamily	70	80 (55-114)	
Apartment	148	43 (34-55)	
Carpeted bedroom floor			
No	146	40 (32-52)	<.001
Yes	124	86 (67-111)	
Report of mold odor			
No	236	56 (46-67)	.74
Yes	33	69 (35-138)	
Report of visible mold			
No	165	57 (46-71)	.94
Yes	104	59 (43-81)	
Report of unrepaired water damage			
No	211	57 (46-70)	.87
Yes	59	59 (41-85)	
Report of leaky pipes			
No	242	50 (49-72)	.32
Yes	26	43 (24-76)	
Report adding water to air			
No	168	60 (48-75)	.54
Yes	102	53 (39-73)	
Wet mop			
No	214	52 (42-63)	.025
Yes	56	86 (57-130)	
Cat in home			
No	236	57 (47-70)	.93
Yes	34	58 (39-88)	
Dog in home			
No	233	55 (45-67)	.28
Yes	37	74 (48-114)	
Season of collection			
Winter	58	60 (43-84)	.49
Spring	71	45 (33-61)	
Summer	99	63 (46-87)	
Fall	42	64 (37-111)	

HAPN, High asthma prevalence neighborhood; *LAPN*, low asthma prevalence neighborhood.
*ANOVA for geometric means.

TABLE E3. Correlation between *A alternata* in bedroom floor dust and home and neighborhood characteristics and allergens

Characteristic	Correlation coefficient*	*P* value
Neighborhood asthma prevalence†	−0.23	<.001
Year home built	−0.12	.058
Median neighborhood household income (500 m) in $10K‡	0.28	<.001
Family household income in $10K	0.21	.001
Neighborhood EC§	−0.12	.043
Der f 1 (μg/g)‖	0.12	.053
Bla g 2 (μg/g)‖	−0.063	.31
Mus m 1 (μg/g)‖	−0.081	.18
Fel d 1 (μg/g)‖	−0.031	.61
Can f 1 (μg/g)‖	<0.001	1.0
Mean relative humidity#	−0.071	.26

*Spearman rank correlation coefficient.
†School-based prevalence of asthma among 5-year-old children for the child's UHF neighborhood (several ZIP codes).
‡Geographical Information Systems census-based variable of the median income of the household in the surrounding radian 500 m.
§Outdoor airborne annual EC based on NYC Community Air Survey.[E6]
‖Allergens measured in bed dust.
#Relative humidity in home measured during the week following dust collection.

TABLE E4. Multivariable model (n = 255)

Characteristic	Beta (95% CI)	*P* value
Apartment building	−0.16 (−0.60 to 0.28)	.48
Carpet	0.58 (0.20 to 0.95)	.003
Wet mop	0.39 (−0.074 to 0.84)	.10
Neighborhood asthma prevalence*	−0.012 (−0.064 to 0.041)	.66
Median neighborhood household income (500 m) in $10K†	0.019 (−0.002 to 0.039)	.073
Family household income in $10K	0.002 (−0.005 to 0.10)	.57

*School-based prevalence of asthma among 5-year-old children for the child's United Hospital Fund Neighborhood (several ZIP codes).
†Geographical Information Systems census-based variable of the median income of the household in the surrounding radian 500 m.

TABLE E5. PR for increased asthma symptoms, higher FENO, and lower FEV_1/FVC among children with asthma with IgE to *A alternata* (≥0.1 IU/mL) (n = 155)

Asthma-related outcome	Frequency among children with asthma	PR for outcome with IgE to *A alternata**
Frequent wheeze (≥4 times past year)	21%	3.7 (1.8-7.8), $P < .001$
Frequent nighttime waking without cold (≥4 times past year)	20%	3.3 (1.6-7.3), $P = .002$
Frequent sleep disturbance by wheeze (≥ once per month)	13%	2.4 (0.97-3.0), $P = .060$
Frequent difficulty breathing (≥4 times past year)	17%	2.8 (1.3-4.0), $P = .011$
Frequent exercise-induced wheeze (≥4 times past year)	11%	2.5 (0.85-3.1), $P = .096$
Urgent medical visits for asthma past year	26%	2.1 (1.1-4.1), $P = .033$
FENO highest quartile (among cases and controls)	31%	2.2 (1.2-4.2), $P = .015$
FEV_1/FVC lowest quartile (among cases and controls)†	28%	2.3 (1.1-4.6), $P = .019$

FVC, Forced vital capacity; *PR*, prevalence ratio.

*In model adjusting for sex, black race, Hispanic ethnicity, maternal asthma, smoker in the home, season, and neighborhood asthma prevalence.

†There were 134 children with valid lung function test.

TABLE E6. Associations* between *A alternata* in bedroom floor dust and frequent wheeze, FENO, and FEV_1/FVC, stratified by neighborhood EC

Asthma-related outcome	Overall	Lower EC	Higher EC	$P_{interaction}$
Asthma (case vs control)	PR = 1.0, P = .97 (n = 269)	PR = 1.0, P = .84 (n = 133)	PR = 1.0, P = .94 (n = 136)	.99
Frequent wheeze among (vs no frequent wheeze among asthma cases)	PR = 1.0, P = .93 (n = 157)	PR = 1.0, P = .86 (n = 74)	PR = 1.1, P = .80 (n = 83)	.72
FENO (among cases and controls)	β = 0.043, P = .078 (n = 269)	β = −0.021, P = .59 (n = 133)	β = 0.089, P = .008 (n = 135)	.028
FEV_1/FVC lowest quartile (among cases and controls)	PR = 1.0, P = .98 (n = 237)	PR = 1.0, P = .95 (n = 113)	PR = 0.99, P = .95 (n = 124)	.99

FVC, Forced vital capacity; *PR*, prevalence ratio.
*Models adjusted for sex, race, Hispanic ethnicity, season, neighborhood asthma prevalence, and dust mite allergen. FENO models were additionally adjusted for ambient NO.

TABLE E7. Association (β coefficient [95% CI])* between indoor allergens and FENO, stratified by neighborhood EC

Allergen (μg/g)†	Lower EC‡	Higher EC‡	$P_{interaction}$§
Der f 1	0.012 (−0.059 to 0.084)	0.068 (−0.009 to 0.15)	.46
Bla g 2	−0.093 (−0.21 to 0.027)	0.094 (0.012 to 0.18)*	.033
Mus m 1	0.018 (−0.036 to 0.073)	0.031 (−0.020 to 0.082)	.43
Fel d 1	−0.022 (−0.094 to 0.049)	0.042 (−0.024 to 0.11)	.22
Can f 1	−0.017 (−0.067 to 0.033)	0.023 (−0.034 to 0.080)	.30

*Liner regression models adjusted for ambient NO, sex, maternal asthma, smoker in home, black race, Hispanic ethnicity, season, and seroatopy.
†Allergen measured in bed dust.
‡Analysis stratified (above and below median) by outdoor airborne annual EC based on NYC Community Air Survey.[E6]
§Interaction term Allergen × Lower or higher EC neighborhood tested in regression model controlling for ambient NO, sex, maternal asthma, smoker in home, black race, Hispanic ethnicity, season, and seroatopy.