

Differential effects of outdoor versus indoor fungal spores on asthma morbidity in inner-city children

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Background: Although sensitization to fungal allergens is prevalent in inner-city children with asthma, the relationship between fungal exposure and morbidity is poorly understood.

Objective: We examined relationships between fungal sensitization, exposure, and asthma morbidity in inner-city children.

Methods: Participants were 5 to 11 years old and enrolled in the Inner-City Asthma Study. This report includes the subset of children with at least 1 positive skin test (PST) response to a fungal allergen extract; for these children, indoor and outdoor airborne culturable fungi levels were measured at baseline and throughout the 2-year study. Asthma morbidity measures were collected prospectively. The primary outcome was symptom days per 2 weeks.

Results: At baseline, children with a PST response to a fungal allergen extract had significantly more symptom days compared with those without a PST response to any fungal allergen extract (6.3 vs 5.7 days per 2 weeks, $P = .04$). During the study, increases in total fungal exposure and indoor *Penicillium* species exposure were associated with increases in symptom days and asthma-related unscheduled visits. Indoor exposures to total fungi and to *Penicillium* species were associated with significant increases in unscheduled visits, even after controlling for outdoor fungal levels. Adverse effects associated with exposure to a specific fungus were stronger among children with PST responses to that fungal allergen extract compared with those seen in children with negative skin test responses.

Conclusion: Outdoor fungal exposure is primarily associated with increased asthma symptoms and increased risk of exacerbations in this population. (J Allergy Clin Immunol 2009;123:1003-1011.)

Key words: Asthma, inner city, airborne fungi, indoor fungi, outdoor fungi, fungal allergens, children

Sensitization to fungal allergens is prevalent in inner-city children with asthma.¹ Although the contributions of allergens, such as cockroach, dust mite, and mouse, have been explored in this population, relatively few studies have focused on relationships between fungal allergen sensitization, exposure, and morbidity. Those reports have found conflicting results. An Institute of Medicine report concluded that although there is evidence of an association between indoor fungi and asthma symptoms in sensitized individuals, there is insufficient evidence of a causal relationship.² Their conclusions are mainly based on studies of self-reported visual mold or dampness rather than measured fungi. Indeed, the relationship between fungal exposure and asthma morbidity remains poorly understood. Cross-sectional studies demonstrate associations between exposure to high concentrations of indoor fungi³⁻⁸ and the presence of asthma or asthma-related measures, such as symptoms or medication use, whereas others⁹⁻¹⁴ have not. Several reports indicate that outdoor fungal exposure is associated with asthma exacerbations,¹⁵ pulmonary function,¹⁶ and medication use,¹⁷ but others^{4,9,18} have not. A 4-year prospective study of inner-city children found that allergen sensitization did not contribute to the seasonal increase in asthma that occurs in the fall (when outdoor fungal concentrations peak) and postulated that another seasonal factor, viral infection, might account for such variation.¹⁹

Given inner-city housing conditions, such as poor ventilation, leaks, and other factors^{1,20,21} that might potentiate problems related to indoor allergens, fungi could be particularly important

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Abbreviations used

DG18: Dichloran glycerol agar
 ICAS: Inner-City Asthma Study
 MSD: Maximum symptom day
 NHLBI: National Heart, Lung, and Blood Institute
 NST: Negative skin test
 OR: Odds ratio
 PST: Positive skin test
 UV: Unscheduled visit

determinants of asthma morbidity for children living in these areas. Because asthma morbidity is disproportionately increased in inner-city children, further investigation of the role of fungi is warranted. Although previous work from the Inner-City Asthma Study (ICAS) assessed home fungal exposure, this study investigates the health effects of fungal exposure in those children sensitized to fungal allergens who participated in the ICAS. Primary outcomes included symptoms and exacerbations, measures of impairment, and risk, as outlined in the National Heart, Lung, and Blood Institute (NHLBI) asthma guidelines.²²

METHODS

The ICAS was a multicenter, randomized controlled trial of environmental intervention to reduce asthma morbidity in which 937 inner-city children aged 5 to 11 years with moderate-to-severe asthma were enrolled. This analysis includes all ICAS participants with a positive skin test (PST) response to at least 1 fungal allergen extract ($n = 469$). All caregivers provided written informed consent. Details regarding recruitment methods, eligibility criteria, and baseline clinical information for participants have been previously published.¹ Indoor and outdoor airborne culturable fungi levels were measured for those children with at least 1 PST response to a fungal allergen extract. The study protocol was approved by the institutional review boards at the participating centers.

Trained staff administered a baseline interview to the primary caretaker. Subsequent asthma morbidity was measured at 2-month intervals over 2 years by telephone. The primary outcome, maximum symptom days (MSDs) per 2 weeks,^{1,23-25} was the largest value among (1) number of days in the past 2 weeks that the child experienced wheezing, chest tightness, or cough; (2) number of nights that the child awoke because of asthma; and (3) number of days that the child had to slow down or discontinue play because of asthma. In addition, the caretaker reported the number of schooldays missed because of asthma, caretaker days of lost sleep within the last 2 weeks, hospitalizations, scheduled and unscheduled clinic visits caused by asthma, and emergency department visits for asthma within the last 2 months. Total asthma-related unscheduled visits (UVs) represented the sum of unscheduled clinic visits and emergency department visits.

During the baseline evaluation, children underwent skin testing (MultiTest II; Lincoln Diagnostics, Decatur, Ill) to histamine and saline controls and 11 allergens, including 4 fungal allergen extracts: *Alternaria alternata*, 1:20 wt/vol; *Cladosporium herbarum*, 1:40 wt/vol; *Penicillium chrysogenum*, 1:20 wt/vol; *Aspergillus* species mix, 1:20 wt/vol (*A flavus*, *A fumigatus*, *A glaucus*, *A nidulans*, and *A niger*); *Dermatophagoides farinae*; *Dermatophagoides pteronyssinus*; cockroach mix (German and American); rat; mouse; cat (standardized 10,000 BAU/mL); and dog (mixed breeds). Extracts were ordered from Greer Laboratories (Lenoir, NC), except for cockroach mix (Bayer Corp, Spokane, Wash). A wheal diameter at least 2 mm larger than that elicited by the negative control was considered positive.

Staff performed home evaluations¹ 1 to 3 weeks after the baseline evaluation and every 6 months for 2 years, for a total of 5 assessments. Indoor and outdoor air samples for fungi were both obtained during each home visit by using a single-stage Burkard Portable Culture Plate Air Sampler (Burkard Manufacturing Co, Rickmansworth, United Kingdom) loaded with a

dichloran glycerol agar (DG18)-filled Petri dish (Remel Laboratories, Lenexa, Kan). The sampling method, which had a well-established precedent, was chosen for its relative ease of analysis.²⁶ DG18 was used because it enables the growth and enumeration of many xerophilic fungi commonly present in homes while not significantly affecting other fungi.²⁶ Bacterial growth was minimized through the use of DG18. The collection time for each sample was 60 seconds (average air volume sampled, 30.5 L). Two consecutive samples were collected outside the participant's home near the main door. If the outdoor temperature was 36°F (2.2°C) or less, outdoor sampling was not performed. Two consecutive samples were collected in the center of the child's bedroom approximately 1 m above the floor. Culture plates were shipped on the day of collection for overnight delivery to a central laboratory. Culture plates were incubated at room temperature (mean, 40 days), and colonies were identified to the genus level, where possible. Results were reported as colony-forming units per cubic meter of air. The methods relating to air-sampling techniques and fungal analyses used in the ICAS have been published elsewhere.²¹

By using a standardized protocol and equipment, separate vacuumed dust samples were collected from the child's bedroom floor and bed. Samples were separated, sealed, and shipped to a central laboratory for allergen measurement (Der p 1, Der f 1, Bla g 1, Fel d 1, Can f 1, and Mus m 1) by means of ELISA with accepted protocols.²⁷⁻²⁹

We used a positive-hole correction³⁰ to correct for the finite number of impaction sites on the plate. (A limited number of impaction sites could adversely affect the number of spores that could be collected.) This positive-hole correction scales total positive colony counts to an estimate of the number of colonies that would have been observed with unlimited impaction sites. Count data were subsequently converted to colony-forming units per cubic meter of air by dividing the corrected colony count by the volume of air sampled.

Single indoor and outdoor values were computed as the mean of the 2 consecutive indoor and outdoor plate values (colony-forming units per cubic meter of air). Correlations between indoor and outdoor concentrations of airborne fungi were analyzed after log₁₀ transformation (after addition of a small constant in light of 0 values) because of highly skewed distributions.

Each 6-month sample was linked to the nearest morbidity follow-up telephone calls surrounding the sample collection date; that is, calls at 4, 6, and 8 months were tied to the 6-month sample. Generalized linear mixed-effect models were fit to predict MSDs and any UVs for asthma. For the analyses, fungi were classified as follows: (1) specific fungus represented an individual genus; (2) the 4 most common fungi combined represented the sum of *Alternaria*, *Aspergillus*, *Cladosporium*, and *Penicillium* species; and (3) total fungi represented the sum of all detectable fungi. For each outcome and type of fungus, 3 separate models were fit: indoor fungal concentration as the morbidity predictor, outdoor concentration as the predictor, and indoor concentration controlling for outdoor concentration. For the last model, both were entered as covariates in the model predicting symptoms; however, we report the effects of indoor concentration and treat outdoor concentration as a nuisance variable. Other fixed effects included environmental intervention group, site, month of the year, bed dust mite allergen levels, and floor cockroach and cat allergen levels. Subject intercepts were included as random effects. For MSDs, a normal distribution with an identity link was used; for any UVs, a binomial distribution with logit link was used, and thus the estimates returned for those outcomes are odds ratios (ORs).

RESULTS

Nine hundred thirty-six children completed the original ICAS intervention study. Fifty percent ($n = 469$) of children had a PST response to at least 1 fungal allergen extract.^{1,21} These children are included in the present study. *Alternaria* species sensitization was most prevalent (36%).²⁴ Sensitization to *Aspergillus*, *Cladosporium*, and *Penicillium* species was found in 27%, 18%, and 13% of children, respectively.²⁴ Indoor and outdoor air sampling were performed in 469 households. Of 4690 possible samples that could have been obtained, 3759 were collected (1799 outdoor and

1960 indoor samples). Characteristics of all ICAS participants, comparing children with negative skin test (NST) responses to any fungal allergen extract with children with PST responses to any fungal allergen extract (those who are included in this study), are presented in Table I. At baseline, children with PST responses to a fungal allergen had significantly higher asthma morbidity compared with children with NST responses to fungal allergens, as reflected by mean MSDs (6.3 vs 5.7 per 2 weeks, $P = .04$). Children with PST responses to a fungal allergen extract were sensitized to more indoor allergens. After adjusting for degree of atopy (defined by total number of PST responses to indoor allergens), we found that degree of atopy did not change the baseline relationship between sensitization to fungal allergens and asthma morbidity. There were no differences between groups in other baseline variables, including exposure to various indoor allergens.

Table II presents baseline correlations between various fungi. Correlations were seen between levels of *Cladosporium* and *Alternaria* species both indoors (0.46) and outdoors (0.49). Weaker correlations were seen between indoor (0.33) and outdoor (0.30) concentrations of *Penicillium* and *Aspergillus* species and outdoor concentrations of *Penicillium* and *Cladosporium* species (0.31).

More than 15 genera of fungi were measurable in inner-city homes, but *Cladosporium*, *Penicillium*, *Aspergillus*, and *Alternaria* species were the most commonly detected.²¹ For the present study, we first looked at total detectable fungi and subsequently focused on the 4 most commonly detected genera. For children sensitized to fungal allergens, the effects of outdoor and indoor fungal exposure on asthma symptoms are presented in Table III. For each 10-fold increase in outdoor exposure to total fungi, there was a statistically significant increase of 1.39 MSDs per 2 weeks ($P < .01$). These findings persisted for outdoor exposure to the combined count of the 4 most common fungi (1.33 days per 2 weeks, $P < .01$). For each 10-fold increase in indoor exposure to total fungi and the 4 most common fungi combined, similar effects on MSDs were seen (1.43 days and 1.32 days per 2 weeks, $P < .01$ for both). After controlling for outdoor exposure, effects of indoor total fungi on MSDs were reduced in magnitude and no longer significant.

Similar analyses were performed for the 4 genera separately. For each 10-fold increase in outdoor exposure, there was a statistically significant effect, with an excess of 1.28 MSDs over 2 weeks for *Alternaria* species ($P < .01$), 1.34 MSDs for *Aspergillus* species ($P = .01$), 1.25 MSDs for *Cladosporium* species ($P < .01$), and 1.42 MSDs for *Penicillium* species ($P < .01$). Similar effects were seen for indoor exposure to the individual genera, but these were no longer significant after controlling for outdoor exposure, except for *Penicillium* species (1.19 MSDs per 2 weeks, $P = .03$).

The relative importance of outdoor versus indoor fungi differed when we analyzed the effect of fungal exposure on asthma exacerbations requiring UVs. As shown in Table III, outdoor fungal exposure had no effect on UVs in the prior 2 months for total fungi, the 4 most common fungi combined, or individual genera, except for *Aspergillus* species (OR, 1.18; 95% CI, 1.01-1.37). In contrast, indoor total fungal exposure was associated with UVs; for each 10-fold increase in total indoor fungi concentration, we found a statistically significant effect (OR, 1.16; 95% CI, 1.02-1.33). For indoor exposure to each of the 4 most common fungi, only *Penicillium* species demonstrated an effect on UVs (OR, 1.13; 95% CI, 1.04-1.24). After adjusting for outdoor fungal exposure, the associations for indoor total fungi and *Penicillium*

species on UVs persisted, and the effect of the 4 most common fungi combined became significant (OR, 1.13; 95% CI, 1.01-1.26).

We examined the health effects associated with exposure to a particular fungal genus among those children who had an NST response to that particular fungal allergen extract. (These children had PST responses to ≥ 1 of the other fungal allergen extracts.) As shown in Table IV, outdoor exposure to *Alternaria* species was associated with health effects among those with NST response to *Alternaria* species. For each 10-fold increase in outdoor *Alternaria* species exposure, *Alternaria* species–nonsensitized children (who were sensitized to ≥ 1 other fungal allergen extracts) experienced an excess of 1.32 MSDs per 2 weeks ($P < .01$). We found similarly significant relationships between increasing *Penicillium* species exposure and MSDs (1.27 days per 2 weeks, $P < .01$) among children with NST responses to *Penicillium* species. Indoors, only *Penicillium* species demonstrated a significant effect on MSDs (1.22 days per 2 weeks, $P < .01$). After controlling for outdoor exposure, we found no effect of indoor exposure to any fungus, including *Penicillium* species, among *Penicillium* species–nonsensitized children.

When we examined UVs, only indoor *Penicillium* species exposure demonstrated an effect for *Penicillium* species–nonsensitized children (OR, 1.11; 95% CI, 1.03-1.20). This finding persisted after controlling for outdoor exposure.

Comparing Tables III and IV, the associations of outdoor and indoor fungal concentrations with symptoms were stronger for specific fungi among subjects with PST responses to fungi as opposed to those with NST responses.

DISCUSSION

This report presents the respiratory health effects of airborne fungi in a sample of atopic inner-city children with moderate-to-severe asthma. Children sensitized to fungal allergens had increased asthma impairment, as defined by the NHLBI asthma guidelines,²² reflected by more symptom days compared with those seen in children with NST responses to fungal allergen extracts. These associations did not change after adjusting for degree of atopy (ie, number of PST responses to indoor allergens). These findings might reflect a distinct effect of fungal allergen sensitization on asthma morbidity and impairment. Outdoor fungal exposure was more strongly related to symptom impairment, whereas indoor exposure appeared to increase exacerbations (as measured based on UVs), an indicator of risk per the NHLBI guidelines.²² During the prospective 2-year study, variability in outdoor exposure to total fungi, including the 4 most commonly recovered genera (*Alternaria*, *Aspergillus*, *Cladosporium*, and *Penicillium* species), was significantly associated with changes in asthma related symptom days. Although we found only a modest increased risk of UVs caused by exposure to fungal spores, such exposure might account for considerable morbidity in this population given the high proportion of children sensitized to fungi. Our findings are consistent with those of Delfino et al.¹⁷ In contrast, a study of inner-city Chicago children found no association between frequency of asthma symptoms and outdoor levels of fungi, which were measured at a single location in the city.⁴ Atopic status was not assessed in the Chicago study, whereas our population consisted only of children sensitized to fungal allergens.

TABLE I. Baseline characteristics*

Characteristic	NST response to fungal allergen extracts (n = 467), mean (SE)	PST response to ≥ 1 fungal allergen extracts (n = 469), mean (SE)	P value
Age (y)	7.7 (0.1)	7.7 (0.1)	.79
MSDs in past 2 wk	5.7 (0.2)	6.3 (0.2)	.04
Schooldays missed in past 2 wk	1.0 (0.1)	1.0 (0.1)	.91
Nights caretaker woke up because of child's asthma in past 2 wk	3.0 (0.2)	3.1 (0.2)	.57
Total unscheduled asthma visits in past 2 mo	0.9 (0.1)	0.9 (0.1)	.73
Hospitalizations for asthma in past 2 mo	0.2 (0.02)	0.2 (0.02)	.46
Average no. of PST responses to other indoor allergens (cat, dog, dust mite, rat, cockroach)	2.2 (1.1)	2.4 (1.4)	.005
Der p 1, bed ($\mu\text{g/g}$)	5.0 (1.0)	2.7 (0.5)	.05
Der f 1, bed ($\mu\text{g/g}$)	3.2 (1.0)	2.7 (0.5)	.61
Bla g 1, floor (U/g)	22.3 (5.1)	23.3 (5.0)	.89
Fel d 1, floor ($\mu\text{g/g}$)	4.6 (1.2)	4.3 (1.1)	.83
Smoker in household	45.4% (2.3%)	51.4% (2.3%)	.07
Randomized to environmental intervention	49.0% (2.3%)	51.2% (2.3%)	.51
Inhaled steroid use	11.1% (1.5%)	11.7% (1.5%)	.78
Male sex	65.1% (2.2%)	60.3% (2.3%)	.13

*Adjusted by the number of PST responses to indoor allergens. Note: there is no change compared with preadjusted data.

TABLE II. Baseline fungal correlations (Pearson correlation and 95% CI)

Site	<i>Alternaria</i> species	<i>Cladosporium</i> species	<i>Aspergillus</i> species	<i>Penicillium</i> species
Indoor				
<i>Alternaria</i> species	1.00	0.46 (0.38 to 0.53)	−0.02 (−0.11 to 0.08)	0.03 (−0.06 to 0.12)
<i>Cladosporium</i> species		1.00	−0.04 (−0.13 to 0.05)	0.19 (0.10 to 0.28)
<i>Aspergillus</i> species			1.00	0.33 (0.24 to 0.41)
<i>Penicillium</i> species				1.00
Outdoor				
<i>Alternaria</i> species	1.00	0.49 (0.41 to 0.56)	0.20 (0.10 to 0.29)	0.03 (−0.07 to 0.13)
<i>Cladosporium</i> species		1.00	0.11 (0.01 to 0.20)	0.31 (0.22 to 0.39)
<i>Aspergillus</i> species			1.00	0.30 (0.21 to 0.39)
<i>Penicillium</i> species				1.00

Individual variations in outdoor exposure to *Aspergillus*, *Cladosporium*, and *Penicillium* species were associated with significant effects on asthma morbidity. These effects were stronger among those who were sensitized and exposed to the individual fungus examined versus those who were sensitized to other fungal allergens, although health effects were observed in this group as well. We did not observe similar effects for *Alternaria* species. A prior publication showed a relationship between *Alternaria* species sensitization, asthma severity, and risk¹⁵ but did not establish an effect of the fungus itself. A cross-sectional study of US homes found indoor *Alternaria* species levels were associated with asthma medication use but not wheezing.³ Allergy skin tests were not performed in that study. It is possible that low variability and relatively low concentrations of *Alternaria* species might explain the lack of effect in our study. *Alternaria* species is a relatively large spore that might not penetrate indoor environments as easily as other fungi. Alternatively, these spores might not remain airborne as long as smaller-spored fungi, especially in relatively still indoor air, so that *Alternaria* species exposure might not have been effectively estimated by using the air-sampling methodology (short-term samples during a period of minimal disturbance) used. Spores might not be a reliable measure of allergen because allergen expression varies over fungal lifecycles and under different environmental conditions.^{31,32} After controlling for outdoor exposure, we found that indoor *Penicillium* species

uniquely affected both symptoms and UVs. Similarly, an association between asthma symptoms and bedroom *Penicillium* species levels was demonstrated in another urban study.⁴ We hypothesize that the differential effects of outdoor versus indoor fungal exposure on symptoms and exacerbations might be related to intensity of exposure; that is, indoor exposure might constitute a more intense exposure of greater duration in a relatively damp, musty, or poorly ventilated environment, as described in inner-city homes,^{1,21} compared with outdoor exposure, which typically occurs for brief periods, thus causing less severe symptoms. However, given the brief collection period and that we did not measure fungi in indoor settled dust (which might be more reflective of indoor exposure), our sampling schema might be biased toward finding outdoor fungi more influential.

We posit several explanations for the association of increased fungal concentrations with increased risk of symptoms among those without sensitization specific to the particular fungal taxon of interest. Because of the positive correlations in concentrations among some of the 4 fungal genera, a concentration increase in a specific fungus to which a subject is not sensitized might correspond with a concentration increase in another fungus to which the subject is sensitized. There might be cross-reactivity among the fungi we studied. Our fungal allergen extracts might not have produced a PST response in subjects who were indeed sensitized. The composition of fungal allergen extracts is

TABLE III. Health effects associated with 10-fold increase* in concentration of fungi among subjects with PST responses to fungal allergen extract

Fungus	No. with fungal allergy†	Excess symptom days per 2 wk associated with increase in outdoor fungi level		Excess symptom days per 2 wk associated with increase in indoor fungi level		Excess symptom days per 2 wk associated with increase in indoor fungi level, controlling for outdoor fungi level	
		Excess symptom days	P value	Excess symptom days	P value	Excess symptom days	P value
Total fungi	469	1.39	<.01	1.43	<.01	1.16	.20
Four most common fungi‡	469	1.33	<.01	1.32	<.01	1.13	.15
<i>Alternaria</i> species	336	1.28	<.01	1.21	.10	0.95	.67
<i>Aspergillus</i> species	253	1.34	.01	1.24	<.01	1.15	.12
<i>Cladosporium</i> species	169	1.25	<.01	1.27	<.01	1.12	.14
<i>Penicillium</i> species	122	1.42	<.01	1.29	<.01	1.19	.03

Fungus	No. with fungal allergy†	UVs in past 2 mo associated with increase in outdoor fungi level§		UVs in past 2 mo associated with increase in indoor fungi level		UVs in past 2 mo associated with increase in indoor fungi level, controlling for outdoor fungi level	
		OR	95% CI	OR	95% CI	OR	95% CI
Total fungi	469	0.96	0.83-1.10	1.16	1.02-1.33	1.22	1.05-1.43
Four most common fungi‡	469	0.99	0.89-1.10	1.09	1.00-1.20	1.13	1.01-1.26
<i>Alternaria</i> species	336	0.99	0.87-1.12	1.02	0.88-1.19	1.03	0.87-1.22
<i>Aspergillus</i> species	253	1.18	1.01-1.37	1.08	0.97-1.19	1.06	0.95-1.19
<i>Cladosporium</i> species	169	1.01	0.93-1.09	1.03	0.95-1.12	1.04	0.94-1.15
<i>Penicillium</i> species	122	1.02	0.90-1.15	1.13	1.04-1.24	1.15	1.05-1.27

*Ten-fold increase as determined by variability in outdoor/indoor measurements over time in a generalized linear mixed-effects model.

†For individual fungal analyses, all subjects in that analysis had a PST response to the specific fungal allergen extract; however, some subjects might be sensitized to the other fungal allergens to which they were skin tested.

‡Sum of *Alternaria*, *Aspergillus*, *Cladosporium*, and *Penicillium* species.

§UVs are emergency department or clinic visits for asthma in the 2 months before the telephone interview. Estimates are ORs.

||Statistically significant.

variable.³³ Also, fungal allergen extracts are not standardized, and therefore there might be inconsistency in their ability to produce a PST response. Alternatively, we skin tested using only a single species for 3 of the 4 fungal genera evaluated, and we might have missed sensitization to other species within each genus (and we did not identify isolates to species level in the environmental samples). Finally, non-IgE-mediated effects of fungi, such as irritant effects, might also explain our findings.

The strengths of our study include the large sample size and appropriate population, atopic children with asthma. We assessed health effects combined with sensitization and exposure data and prospective evaluation of asthma outcomes rather than retrospective self-report. We used a prospective, longitudinal study design of 2 years' duration. Both indoor and outdoor exposures were taken into account, and all environmental sampling was conducted in duplicate.

Although our study is the largest examining the effects of fungi on asthma in inner-city children, we acknowledge that it has limitations. The designation of 2-mm wheal size as evidence of sensitization might be criticized as inadequate. However, when we performed the same analysis for those subjects with a wheal size of 3 mm or greater, our results did not significantly change, except for *Cladosporium* species, for which indoor exposure was also associated with an increase in MSDs.

Home measurements were only performed for those participants with PST responses to fungal allergen extracts. Consequently, we lack the ability to compare our findings regarding exposure or morbidity with those in children who were not sensitized to fungal allergens.

Because all subjects were reactive to at least 1 fungal allergen extract, we cannot determine whether the significant health effects are due to cross-reactivity versus non-IgE (irritant, toxicogenic, and other) effects. This possibility is supported by the similar magnitude of the ORs for UVs in the PST and NST response groups.

The short sampling time and inherent variability between consecutive samples might not accurately reflect exposure over long time periods. However, it is unlikely that our sampling method would result in a spurious outcome. A failure to find any relationship between exposure and symptoms would be more likely.

Other fungi might affect asthma morbidity, but our limited skin test panel precluded us from examining their effects. The use of culture for fungal recoveries (compared, for example, with direct spore counting) limits the numbers and types of fungi recovered.

A recent study found that *Alternaria* species antigens are measurable, even in the absence of culturable *Alternaria* species.³⁴ These antigens might have been associated with nonculturable spores. Currently available methods for estimating concentrations of nonculturable spores also have limitations. Direct spore counting does not have the specificity of culture or quantitative PCR, but it enables the detection of the widest variety of spore types, including basidiospores and obligate plant pathogens, among many other potentially allergenic types. Quantitative PCR is limited by the number of fungal primers currently available, most of which are species specific and not pangeneric, and the assay can also be inhibited by a variety of chemicals, including components of airborne particulate matter.^{35,36} We performed identification to

TABLE IV. Health effects associated with 10-fold increase* in concentration of fungi among children with NST responses to a particular fungal allergen extract

Fungus	No. nonallergic†	Excess symptom days per 2 wk associated with increase in outdoor fungi level		Excess symptom days per 2 wk associated with increase in indoor fungi level		Excess symptom days per 2 wk associated with increase in indoor fungi level, controlling for outdoor fungi level	
		Excess symptom days	P value	Excess symptom days	P value	Excess symptom days	P value
<i>Alternaria</i> species	133	1.32	.01	1.23	.10	0.99	.97
<i>Aspergillus</i> species	216	1.23	.09	1.14	.11	1.06	.52
<i>Cladosporium</i> species	300	1.12	.06	1.10	.08	0.97	.72
<i>Penicillium</i> species	347	1.27	<.01	1.22	<.01	1.13	.06

Fungus	No. nonallergic†	UVs in past 2 mo associated with increase in outdoor fungi level‡		UVs in past 2 mo associated with increase in indoor fungi level		UVs in past 2 mo associated with increase in indoor fungi level, controlling for outdoor fungi level	
		OR	95% CI	OR	OR	95% CI	OR
<i>Alternaria</i> species	133	1.03	0.90-1.18	1.06	0.90-1.25	1.07	0.89-1.29
<i>Aspergillus</i> species	216	1.16	0.99-1.36	1.05	0.95-1.16	1.05	0.94-1.17
<i>Cladosporium</i> species	300	0.97	0.90-1.05	1.01	0.93-1.09	1.01	0.91-1.11
<i>Penicillium</i> species	347	0.99	0.88-1.10	1.11§	1.03-1.20	1.12§	1.03-1.22

*Ten-fold increase as determined by variability in outdoor/indoor measurements over time in a generalized linear mixed effect model.

†For individual fungal analyses, all subjects in that analysis had NST responses to the given fungal allergen extract; however, some subjects might be sensitized to the other fungal allergens to which they were skin tested.

‡UVs are emergency department or clinic visits for asthma.

§Statistically significant.

the genus level and not to the species level; more specific identifications might have highlighted additional indoor/outdoor differences. We did not assess copollutants, which might affect responses to fungal allergens.

Our findings suggest that fungal allergen sensitization and exposure independently contribute to asthma morbidity in inner-city children with asthma and that these effects are related to exposure to outdoor fungi and indoor *Penicillium* species. The data support the findings of a study that demonstrated an increase in UVs and the potential value of home remediation interventions in the inner city.³⁷ The results of our study identify new possibilities for future environmental intervention strategies for this population.

Clinical implications: Outdoor and indoor fungi, particularly *Penicillium* species, worsen asthma morbidity in inner-city children. Fungal exposure should be considered a potential cause of poor asthma control in this population.

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