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

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Abstract

Background—While 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 5–11 years old and enrolled in the Inner-City Asthma Study. This report includes the subset of children with at least 1 positive skin test to a fungal allergen extract; for these children, indoor and outdoor airborne culturable fungi 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.

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Results—At baseline, children with a positive skin test to a fungal allergen extract had significantly more symptom days compared to those without positive skin test to any fungal allergen extract (6.3 vs 5.7 days per 2 weeks, p=0.04). During the study, elevations in total fungal exposure and indoor *Penicillium* exposure were associated with increases in symptom days and asthma unscheduled visits. Indoor exposures to total fungi and to *Penicillium* 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 positive skin test to that fungal allergen extract compared to skin test negative children.

Conclusion—Outdoor fungal exposure is primarily associated with increased asthma symptoms and increased risk of exacerbations in this population.

Keywords

asthma; inner city; airborne fungi; indoor fungi; outdoor fungi; fungal allergens; children

INTRODUCTION

Sensitization to fungal allergens is prevalent in inner-city children with asthma.[1] While 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 while 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 fungi[3–8] and presence of asthma or asthma related measures such as symptoms or medication use while others[9–14] 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 four year prospective study of inner-city children found that allergen sensitization did not contribute to the seasonal increase in asthma which occurs in the fall (when outdoor fungal concentrations peak) and postulated that another seasonal factor, viral infection, may account for such variation.[19]

Given inner-city housing conditions such as poor ventilation, leaks and other factors[1, 20, 21] which may potentiate problems related to indoor allergens, fungi may be particularly important determinants of asthma morbidity for children living in these areas. Since asthma morbidity is disproportionately increased in inner-city children, further investigation of the role of fungi is warranted. While 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 ICAS. Primary outcomes included symptoms and exacerbations, measures of impairment and risk as outlined in the NHLBI asthma guidelines.[22]

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METHODS

ICAS was a multi-center randomized controlled trial of environmental intervention to reduce asthma morbidity in which 937 inner-city children aged 5–11 years with moderate to severe asthma were enrolled. This analysis includes all ICAS participants with a positive skin test (PST) to at least 1 fungal allergen extract (n=467). 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 were measured for those children with at least 1 PST 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 via telephone. The primary outcome, maximum symptom days (MSD) 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 school days missed due to asthma, caretaker days of lost sleep within the last 2 weeks, hospitalizations, scheduled and unscheduled clinic visits due to asthma, and emergency department (ED) visits for asthma within the last 2 months. Total asthma unscheduled visits represented the sum of unscheduled clinic visits and ED visits.

During the baseline evaluation, children underwent skin testing (MultiTest II, Lincoln Diagnostics, Decatur, IL) to histamine and saline controls and 11 allergens, including 4 fungal allergen extracts: *Alternaria alternata 1:20 w/v*, *Cladosporium herbarum 1:40 w/v*, *Penicillium chrysogenum 1:20 w/v*, *Aspergillus* mix *1:20 w/v* (*A. flavus, A. fumigatus, A. glaucus, A. nidulans, 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 Corporation, Spokane, WA). A wheal diameter ≥2mm larger than the negative control was considered positive.

Staff performed home evaluations[1] one to three weeks after the baseline evaluation and every 6 months for 2 years, for a total of five assessments. Indoor and outdoor air samples for fungi were both obtained during each home visit 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, Kansas). 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 impacting 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 liters). 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 lower, outdoor sampling was not performed. Two consecutive samples were collected in the center of the child's bedroom approximately

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1 meter 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 (CFU/m^3). The methods relating to air sampling techniques and fungal analyses used in ICAS have been published elsewhere.[21]

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 ELISA using accepted protocols.[27–29]

Data Analysis and Statistical Methods

We used a positive hole correction[30] 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 CFU/m^3 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 (CFU/m^3). Correlations between indoor and outdoor concentrations of airborne fungi were analyzed after $\log_{10}$ transformation (after addition of a small constant in light of 0 values) because of highly skewed distributions.

Each six-month sample was linked to the nearest morbidity follow-up phone calls surrounding the sample collection date, i.e. calls at 4, 6 and 8 months were tied to the 6 month sample. Generalized linear mixed effect models were fit to predict MSD and any unscheduled visits for asthma. For the analyses, fungi were classified as follows: 1) specific fungus represented an individual genus, 2) 4 most common fungi combined represented the sum of *Alternaria, Aspergillus, Cladosporium and Penicillium,* and 3) total fungi represented the sum of all detectable fungi. For each outcome and type of fungus, three separate models were fit: indoor fungal concentration as the morbidity predictor, outdoor concentration as the predictor and indoor concentration controlling for outdoor. For the last model, both were entered as covariates in the model predicting symptoms, however, we report the effects of indoor and treat the outdoor 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 MSD a normal distribution with an identity link was used; for any unscheduled visits a binomial distribution with logit link was used, thus the estimates returned for those outcomes are odds ratios.

RESULTS

Nine hundred thirty-six children completed the original ICAS intervention study. Fifty percent (n=469) of children had PST to at least one fungal allergen extract.[1,21] These children are included in the present study. *Alternaria* sensitization was most prevalent,

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(36%).[24] Sensitization to *Aspergillus*, *Cladosporium* and *Penicillium* was found in 27%, 18% and 13% of children, respectively.[24] Indoor and outdoor air sampling were performed in 469 households. Out of 4690 possible samples that could have been obtained, 3759 were collected (1799 outdoor and 1960 indoor). Characteristics of all ICAS participants, comparing children with negative skin test (NST) to any fungal allergen extract to children with PST to any fungal allergen extract (those who are included in this study) are presented in Table I. At baseline, children with PST to a fungal allergen had significantly higher asthma morbidity compared to children with NST to fungal allergens as reflected by mean MSD (6.3 vs 5.7 per 2 weeks, p=0.04). Children with PST to a fungal allergen extract were sensitized to more indoor allergens. After adjusting for degree of atopy (defined by total number of PSTs 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* 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* and outdoor concentrations of *Penicillium* and *Cladosporium* (0.31).

Over 15 genera of fungi were measurable in inner-city homes but *Cladosporium*, *Penicillium*, *Aspergillus* and *Alternaria* were the most commonly detected.[21] For the present study, we first looked at total detectable fungi and subsequently focused on the four 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 MSD per 2 weeks ($p<0.01$). These findings persisted for outdoor exposure to the combined count of the 4 most common fungi (1.33 days per 2 weeks, $p<0.01$). For each 10-fold increase in indoor exposure to total fungi and the 4 most common fungi combined, similar effects on MSD were seen (1.43 days and 1.32 days per 2 weeks, $p<0.01$ for both). After controlling for outdoor exposure, effects of indoor total fungi on MSD 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 MSD over 2 weeks for *Alternaria* ($p<0.01$), 1.34 MSD for *Aspergillus* ($p=0.01$), 1.25 MSD for *Cladosporium* ($p<0.01$) and 1.42 MSD for *Penicillium* ($p<0.01$). 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* (1.19 MSD per 2 weeks, $p=0.03$).

The relative importance of outdoor versus indoor fungi differed when we analyzed the effect of fungal exposure on asthma exacerbations requiring unscheduled visits (UV). As shown in Table III, outdoor fungal exposure had no effect on UV in the prior 2 months for total fungi, the 4 most common fungi combined or individual genera except for *Aspergillus* (OR, 95% CI = 1.18, 1.01–1.37). In contrast, indoor total fungal exposure was associated with UV; for

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each 10-fold increase in total indoor fungi, we found a statistically significant effect (OR, 95% CI = 1.16, 1.02–1.33). For indoor exposure to each of the 4 most common fungi, only *Penicillium* demonstrated an effect on UV (OR, 95% CI = 1.13, 1.04–1.24). After adjusting for outdoor fungal exposure, the associations for indoor total fungi and *Penicillium* on UV persisted, and the effect of the 4 most common fungi combined became significant (OR, 95% CI = 1.13, 1.01–1.26).

We examined the health effects associated with exposure to a particular fungal genus among those children who had a NST to that particular fungal allergen extract (these children had PST to one or more of the other fungal allergen extracts). As shown in Table IV, outdoor exposure to *Alternaria* was associated with health effects among those with NST to *Alternaria*. For each 10-fold increase in outdoor *Alternaria* exposure, *Alternaria* non-sensitized children (who were sensitized to one or more other fungal allergen extracts) experienced an excess of 1.32 MSD per 2 weeks ($p<0.01$). We found similarly significant relationships between increasing *Penicillium* exposure and MSD (1.27 days per 2 weeks, $p<0.01$), among children with NST to *Penicillium*. Indoors, only *Penicillium* demonstrated significant effect on MSD (1.22 days per 2 weeks, $p<0.01$). After controlling for outdoor exposure, we found no effect of indoor exposure to any fungus, including *Penicillium*, among *Penicillium* non-sensitized children.

When we examined UV, only indoor *Penicillium* exposure demonstrated an effect for *Penicillium* non-sensitized children (OR, 95% CI = 1.11, 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 to fungi as opposed to those with NST.

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 to children with NST to fungal allergen extracts. These associations did not change after adjusting for degree of atopy (i.e. number of PST to indoor allergens). These findings may reflect a distinct effect of fungal allergen sensitization on asthma morbidity and impairment. Outdoor fungal exposure was more strongly related to symptom impairment while indoor exposure appeared to increase exacerbations (as measured by unscheduled visits), 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*), was significantly associated with changes in asthma related symptom days. While we found only a modest increased risk of UV due to exposure to fungal spores, such exposure may 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

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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.

Individual variations in outdoor exposure to *Aspergillus*, *Cladosporium* and *Penicillium* 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*. A prior publication showed a relationship between *Alternaria* sensitization, asthma severity and risk[15] but did not establish an effect of the fungus itself. A cross-sectional study of US homes found indoor *Alternaria* 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 relative low concentrations of *Alternaria* may explain the lack of effect in our study. *Alternaria* is a relatively large spore that may not penetrate indoor environments as easily as other fungi. Alternatively, these spores may not remain airborne as long as smaller-spored fungi, especially in relatively still indoor air, so that *Alternaria* exposure may not have been effectively estimated using the air sampling methodology (short-term samples during a period of minimal disturbance) employed. Spores may not be a reliable measure of allergen, since allergen expression varies over fungal life cycles and under different environmental conditions.[31, 32] After controlling for outdoor exposure, we found that indoor *Penicillium* uniquely affected both symptoms and UV. Similarly, an association between asthma symptoms and bedroom *Penicillium* levels was demonstrated in another urban study.[4] We hypothesize that the differential effects of outdoor versus indoor fungal exposure on symptoms and exacerbations may be related to intensity of exposure, i.e. indoor exposure may 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 to 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 may be more reflective of indoor exposure) our sampling schema may be biased towards finding outdoor fungi more influential.

We posit several explanations for the association of elevated fungal concentrations with increased risk of symptoms amongst those without sensitization specific to the particular fungal taxon of interest. Due to the positive correlations in concentrations among some of the four 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 may be cross-reactivity among the fungi we studied. Our fungal allergen extracts may not have produced a PST in subjects who were indeed sensitized. The composition of fungal allergen extracts is variable.[33] Also, fungal allergen extracts are not standardized, so there may be inconsistency in their ability to produce a PST. Alternatively, we skin tested using only a single species for 3 of the 4 fungal genera evaluated, and we may 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, may also explain our findings.

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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 employed a prospective, longitudinal study design of two years. Both indoor and outdoor exposures were taken into account and all environmental sampling was conducted in duplicate.

While our study is the largest study 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 may 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*, for which indoor exposure was also associated with increase in MSD. Home measurements were only performed on those participants with PST to fungal allergen extracts. Consequently, we lack the ability to compare our findings regarding exposure or morbidity to children who were not sensitized to fungal allergens. Since 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, toxigenic, other) effects. This possibility is supported by the similar magnitude of the odds ratios for UV in the PST and NST groups. The short sampling time and inherent variability between consecutive samples may 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 may 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, to direct spore counting) limits the numbers and types of fungi recovered. A recent study found that *Alternaria* antigens are measurable even in the absence of culturable *Alternaria*.[34] These antigens might have been associated with non-culturable spores. Currently available methods for estimating concentrations of non-culturable 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 not pan-generic, and the assay can also be inhibited by a variety of chemicals, including components of airborne particulate matter.[35, 36] We performed identification to the genus level, not to species; more specific identifications might have highlighted additional indoor/outdoor differences. We did not assess co-pollutants, which may 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*. The data support the findings of a study which demonstrated an increase in unscheduled visits 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.

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Abbreviations

ICAS	Inner-City Asthma Study
NHLBI	National Heart, Lung and Blood Institute
ED	emergency department
MSD	maximum symptoms days
CFU	colony forming units
ELISA	enzyme-linked immunosorbent assay
NST	negative skin test
PST	positive skin test
UV	unscheduled visit
PCR	polymerase chain reaction

References

1. Crain EF, Walter M, O'Connor GT, Mitchell H, Gruchalla RS, Kattan M, et al. Home and allergic characteristics of children with asthma in seven U.S. urban communities and design of an environmental intervention: the Inner-City Asthma Study. Environ Health Perspect. 2002; 110:939–45. [PubMed: 12204830]
2. Health CoDISa. Damp Indoor Spaces and Health. Washington (DC): The National Academies Press; 2004.
3. Salo PM, Arbes SJ Jr, Sever M, Jaramillo R, Cohn RD, London SJ, et al. Exposure to Alternaria alternata in US homes is associated with asthma symptoms. J Allergy Clin Immunol. 2006; 118:892–8. [PubMed: 17030243]
4. Turyk M, Curtis L, Scheff P, Contraras A, Coover L, Hernandez E, et al. Environmental allergens and asthma morbidity in low-income children. J Asthma. 2006; 43:453–7. [PubMed: 16952864]
5. Su HJ, Rotnitzky A, Burge HA, Spengler JD. Examination of fungi in domestic interiors by using factor analysis: correlations and associations with home factors. Appl Environ Microbiol. 1992; 58:181–6. [PubMed: 1539973]
6. Platt SD, Martin CJ, Hunt SM, Lewis CW. Damp housing, mould growth, and symptomatic health state. Bmj. 1989; 298:1673–8. [PubMed: 2503174]
7. Waegemaekers M, Van Wageningen N, Brunekreef B, Boleij JS. Respiratory symptoms in damp homes. A pilot study. Allergy. 1989; 44:192–8. [PubMed: 2712255]
8. Strachan DP, Flannigan B, McCabe EM, McGarry F. Quantification of airborne moulds in the homes of children with and without wheeze. Thorax. 1990; 45:382–7. [PubMed: 2382244]
9. Li CS, Hsu LY, Chou CC, Hsieh KH. Fungus allergens inside and outside the residences of atopic and control children. Arch Environ Health. 1995; 50:38–43. [PubMed: 7717768]
10. Verhoeff AP, van Wijnen JH, van Reenen-Hoekstra ES, Samson RA, van Strien RT, Brunekreef B. Fungal propagules in house dust. II. Relation with residential characteristics and respiratory symptoms. Allergy. 1994; 49:540–7. [PubMed: 7825721]
11. Gent JF, Ren P, Belanger K, Triche E, Bracken MB, Holford TR, et al. Levels of household mold associated with respiratory symptoms in the first year of life in a cohort at risk for asthma. Environ Health Perspect. 2002; 110:A781–6. [PubMed: 12460818]
12. Belanger K, Beckett W, Triche E, Bracken MB, Holford T, Ren P, et al. Symptoms of wheeze and persistent cough in the first year of life: associations with indoor allergens, air contaminants, and maternal history of asthma. Am J Epidemiol. 2003; 158:195–202. [PubMed: 12882940]

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13. Wood RA, Eggleston PA, Lind P, Ingemann L, Schwartz B, Graveson S, et al. Antigenic analysis of household dust samples. Am Rev Respir Dis. 1988; 137:358–63. [PubMed: 3341627]
14. Wickman M, Gravesen S, Nordvall SL, Pershagen G, Sundell J. Indoor viable dust-bound microfungi in relation to residential characteristics, living habits, and symptoms in atopic and control children. J Allergy Clin Immunol. 1992; 89:752–9. [PubMed: 1545096]
15. O'Hollaren MT, Yunginger JW, Offord KP, Somers MJ, O'Connell EJ, Ballard DJ, et al. Exposure to an aeroallergen as a possible precipitating factor in respiratory arrest in young patients with asthma. N Engl J Med. 1991; 324:359–63. [PubMed: 1987459]
16. Beaumont F, Kauffman HF, Sluiter HJ, De Vries K. Sequential sampling of fungal air spores inside and outside the homes of mould-sensitive, asthmatic patients: a search for a relationship to obstructive reactions. Ann Allergy. 1985; 55:740–6. [PubMed: 4061983]
17. Delfino RJ, Coate BD, Zeiger RS, Seltzer JM, Street DH, Koutrakis P. Daily asthma severity in relation to personal ozone exposure and outdoor fungal spores. Am J Respir Crit Care Med. 1996; 154:633–41. [PubMed: 8810598]
18. Holst PE, Coleman ED, Sheridan JE, O'Donnell TV, Sutthoff PT. Asthma and fungi in the home. N Z Med J. 1983; 96:718–20. [PubMed: 6577349]
19. Gergen PJ, Mitchell H, Lynn H. Understanding the seasonal pattern of childhood asthma: results from the National Cooperative Inner-City Asthma Study (NCICAS). J Pediatr. 2002; 141:631–6. [PubMed: 12410190]
20. Kattan M, Mitchell H, Eggleston P, Gergen P, Crain E, Redline S, et al. Characteristics of inner-city children with asthma: the National Cooperative Inner-City Asthma Study. Pediatr Pulmonol. 1997; 24:253–62. [PubMed: 9368259]
21. O'Connor GT, Walter M, Mitchell H, Kattan M, Morgan WJ, Gruchalla RS, et al. Airborne fungi in the homes of children with asthma in low-income urban communities: The Inner-City Asthma Study. J Allergy Clin Immunol. 2004; 114:599–606. [PubMed: 15356564]
22. Expert Panel Report 3 (EPR-3): Guidelines for the Diagnosis and Management of Asthma-Summary Report 2007. J Allergy Clin Immunol. 2007; 120:S94–138. [PubMed: 17983880]
23. Evans R 3rd, Gergen PJ, Mitchell H, Kattan M, Kercsmar C, Crain E, et al. A randomized clinical trial to reduce asthma morbidity among inner-city children: results of the National Cooperative Inner-City Asthma Study. J Pediatr. 1999; 135:332–8. [PubMed: 10484799]
24. Gruchalla RS, Pongracic J, Plaut M, Evans R 3rd, Visness CM, Walter M, et al. Inner City Asthma Study: relationships among sensitivity, allergen exposure, and asthma morbidity. J Allergy Clin Immunol. 2005; 115:478–85. [PubMed: 15753892]
25. Morgan WJ, Crain EF, Gruchalla RS, O'Connor GT, Kattan M, Evans R 3rd, et al. Results of a home-based environmental intervention among urban children with asthma. N Engl J Med. 2004; 351:1068–80. [PubMed: 15356304]
26. Hoekstra, E.; Samson, R.; Summerbell, RC. Methods for the detection and isolation of fungi in the indoor environments. In: Samson, RA.; Hoekstra, ES., editors. Introduction to food- and airborne fungi ed. Centraalbureau voor Schimmelcultures; Utrecht, Netherlands: 2004.
27. Chapman MD, Heymann PW, Wilkins SR, Brown MJ, Platts-Mills TA. Monoclonal immunoassays for major dust mite (Dermatophagoides) allergens, Der p I and Der f I, and quantitative analysis of the allergen content of mite and house dust extracts. J Allergy Clin Immunol. 1987; 80:184–94. [PubMed: 3611539]
28. Ingram JM, Sporik R, Rose G, Honsinger R, Chapman MD, Platts-Mills TA. Quantitative assessment of exposure to dog (Can f 1) and cat (Fel d 1) allergens: relation to sensitization and asthma among children living in Los Alamos, New Mexico. J Allergy Clin Immunol. 1995; 96:449–56. [PubMed: 7560654]
29. Pollart SM, Mullins DE, Vailes LD, Hayden ML, Platts-Mills TA, Sutherland WM, et al. Identification, quantitation, and purification of cockroach allergens using monoclonal antibodies. J Allergy Clin Immunol. 1991; 87:511–21. [PubMed: 1993811]
30. Andersen AA. New sampler for the collection, sizing, and enumeration of viable airborne particles. J Bacteriol. 1958; 76:471–84. [PubMed: 13598704]

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31. Arruda LK, Mann BJ, Chapman MD. Selective expression of a major allergen and cytotoxin, Asp f I, in Aspergillus fumigatus. Implications for the immunopathogenesis of Aspergillus-related diseases. J Immunol. 1992; 149:3354–9. [PubMed: 1431110]

32. Little SA, Longbottom JL, Warner JO. Optimized preparation of Aspergillus fumigatus extracts for allergy diagnosis. Clin Exp Allergy. 1993; 23:835–42. [PubMed: 10780890]

33. Esch RE. Manufacturing and standardizing fungal allergen products. J Allergy Clin Immunol. 2004; 113:210–5. [PubMed: 14767431]

34. Peters JL, Muilenberg ML, Rogers CA, Burge HA, Spengler JD. Alternaria measures in inner-city, low-income housing by immunoassay and culture-based analysis. Ann Allergy Asthma Immunol. 2008; 100:364–9. [PubMed: 18450123]

35. Cruz P, Buttner MP. Development and evaluation of a real-time quantitative PCR assay for Aspergillus flavus. Mycologia. 2008; 100:683–90. [PubMed: 18959152]

36. McDevitt J, Lees P, Merz W, Schwab K. Inhibition of quantititative PCT analysis of fungal conidia associated with indoor air particulate matter. Aerobiologia. 2007; 23:35–45.

37. Kercsmar CM, Dearborn DG, Schluchter M, Xue L, Kirchner HL, Sobolewski J, et al. Reduction in asthma morbidity in children as a result of home remediation aimed at moisture sources. Environ Health Perspect. 2006; 114:1574–80. [PubMed: 17035145]

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Clinical implications

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

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Table I

Baseline characteristics*

Characteristic	Skin test negative to fungal allergen extracts (N=467) Mean (Std. Err.)	Skin test positive to ≥1 fungal allergen extracts (N=469) Mean (Std. Err.)	P-Value
Age (Years)	7.7 (0.1)	7.7 (0.1)	0.79
Maximum symptom days in the past 2 weeks	5.7 (0.2)	6.3 (0.2)	0.04
School days missed in past 2 weeks	1.0 (0.1)	1.0 (0.1)	0.91
Nights caretaker woke up due to child's asthma in past 2 weeks	3.0 (0.2)	3.1 (0.2)	0.57
Total unscheduled asthma visits in past 2 months	0.9 (0.1)	0.9 (0.1)	0.73
Hospitalizations for asthma in past 2 months	0.2 (0.02)	0.2 (0.02)	0.46
Average # of positive skin tests to other indoor allergens (cat, dog, dust mite, rat, cockroach)	2.2 (1.1)	2.4(1.4)	0.005
Der p 1 Bed (μg/g)	5.0 (1.0)	2.7 (0.5)	0.05
Der f 1 Bed (μg/g)	3.2 (1.0)	2.7 (0.5)	0.61
Bla g 1 Floor (U/g)	22.3 (5.1)	23.3 (5.0)	0.89
Fel d 1 Floor (μg/g)	4.6 (1.2)	4.3 (1.1)	0.83
Smoker in household randomized to environmental intervention	45.4% (2.3)	51.4% (2.3)	0.07
	49.0% (2.3)	51.2% (2.3)	0.51
Inhaled steroid use	11.1% (1.5)	11.7% (1.5)	0.78
Male	65.1%(2.2)	60.3% (2.3)	0.13

*Adjusted by # of positive skin tests to indoor allergens (note: no change compared to pre-adjusted data)

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Table II

Baseline fungal correlations (Pearson correlation and 95% confidence interval)

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

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Table III

Health effects associated with 10-fold increase[§] in concentration of fungi among subjects with positive skin test to fungal allergen extract

		Excess symptom days per two weeks associated with increase in outdoor fungi		Excess symptom days per two weeks associated with increase in indoor fungi		Excess symptom days per two weeks associated with increase in indoor fungi, controlling for outdoor	
Fungus	**Number with fungal allergy [2]**	**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	0.20
Four most common fungi[1]	469	1.33	<.01	1.32	<.01	1.13	0.15
Alternaria	336	1.28	<.01	1.21	0.10	0.95	0.67
Aspergillus	253	1.34	0.01	1.24	<.01	1.15	0.12
Cladosporium	169	1.25	<.01	1.27	<.01	1.12	0.14
Penicillium	122	1.42	<.01	1.29	<.01	1.19	0.03
		UV in past 2 months associated with increase in outdoor fungi[3]		**UV in past 2 months associated with increase in indoor fungi**		**UV in past 2 months associated with increase in indoor fungi, controlling for outdoor**	
		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[1]	469	0.99	0.89, 1.10	1.09*	1.00, 1.20	1.13*	1.01, 1.26
Alternaria	336	0.99	0.87, 1.12	1.02	0.88, 1.19	1.03	0.87, 1.22
Aspergillus	253	1.18*	1.01, 1.37	1.08	0.97, 1.19	1.06	0.95, 1.19
Cladosporium	169	1.01	0.93, 1.09	1.03	0.95, 1.12	1.04	0.94, 1.15
Penicillium	122	1.02	0.90, 1.15	1.13*	1.04, 1.24	1.15*	1.05, 1.27

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

[1] Sum of *Alternaria, Aspergillus, Cladosporium*, and *Penicillium*

[2] For individual fungal analyses, all subjects in that analysis had a positive skin test to the specific fungal allergen extract, however, some subjects may be sensitized to the other fungal allergens to which they were skin tested.

[3] UV=Unscheduled visits to emergency department or clinic for asthma in the 2 months prior to the telephone interview; Estimates are odds ratios

* denotes statistically significant

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Table IV

Health effects associated with 10-fold increase[§] in concentration of fungi among children with negative skin test to a particular fungal allergen extract

		Excess symptom days per two weeks associated with increase in outdoor fungi		Excess symptom days per two weeks associated with increase in indoor fungi		Excess symptom days per two weeks associated with increase in indoor fungi, controlling for outdoor	
Fungus	**Number non-allergic [1]**	**Excess symptom days**	**P value**	**Excess symptom days**	**P value**	**Excess symptom days**	**Pvalue**
Alternaria	133	1.32	0.01	1.23	0.10	0.99	0.97
Aspergillus	216	1.23	0.09	1.14	0.11	1.06	0.52
Cladosporium	300	1.12	0.06	1.10	0.08	0.97	0.72
Penicillium	347	1.27	<.01	1.22	<.01	1.13	0.06
		UV in past 2 months associated with increase in outdoor fungi [2]		**UV in past 2 months associated with increase in indoor fungi**		**UV in past 2 months associated with increase in indoor fungi, controlling for outdoor**	
		OR	**95% CI**	**OR**	**OR**	**95% CI**	**OR**
Alternaria	133	1.03	0.90, 1.18	1.06	0.90, 1.25	1.07	0.89, 1.29
Aspergillus	216	1.16	0.99, 1.36	1.05	0.95, 1.16	1.05	0.94, 1.17
Cladosporium	300	0.97	0.90, 1.05	1.01	0.93, 1.09	1.01	0.91, 1.11
Penicillium	347	0.99	0.88, 1.10	1.11*	1.03, 1.20	1.12*	1.03, 1.22

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

[1] For individual fungal analyses, all subjects in that analysis had negative skin test to the given fungal allergen extract, however, some subjects may be sensitized to the other fungal allergens to which they were skin tested.

[2] UV=Unscheduled visits to emergency department or clinic for asthma.

* denotes statistically significant