

Association between indoor mold and asthma among children in Buffalo, New York

Abstract Asthma is a leading chronic disease among children and places a significant burden on public health. Exposure to indoor mold has been associated with asthma symptoms. However, many mold assessments have relied on visual or other identification of damp conditions and mold presence, thus have not examined associations with specific fungal genera. The objective of this case–control study was to examine the relationship between airborne mold concentrations and asthma status among children and to identify the contribution from specific mold genera in air. Participants completed a questionnaire of home environmental conditions and underwent indoor air sampling in the home, from which viable and total-count fungal spores were quantified. The most prevalent fungi in the homes were the allergenic molds *Cladosporium* (98% and 87% of homes from viable and total count samples, respectively) and *Penicillium* (91% and 73%). There were no significant differences in mean fungal concentrations between the homes of cases and controls, although the observed rate of exposure to several molds was higher among the cases. Among children who lacked a family history of asthma, cases had significantly higher exposures to viable *Aspergillus*. Measured humidity levels in the home corresponded with some self-reported indicators of mold and dampness.

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Practical Implications

The results of this study support existing literature that indoor fungal exposures play a role in current asthma status and that some qualitative assessments of mold exposure correspond to fungi present in indoor air.

Introduction

Each year, millions of children suffer from respiratory symptoms such as wheeze, cough and shortness of breath. Symptomatic asthma is a suspected cause of activity limitation (National Center for Health Statistics, 2006) and missed school days (Silverstein et al., 2001). Despite recent trends suggesting that asthma rates have begun to decline, (Akinbami and Schoendorf, 2002; Akinbami et al., 2009; Pearce and Douwes, 2005) asthma remains the leading chronic illness affecting children in the United States, where 9% of children under 15 years old have doctor-diagnosed asthma (Heyman et al., 2008).

Children who live in homes with dampness or mold are at increased risk for lower respiratory symptoms

and asthma hospitalizations (Belanger et al., 2003; Wever-Hess et al., 2000). Two different Institute of Medicine panels concluded there is sufficient evidence of an association between indoor fungi exposure and asthma symptoms in sensitized patients with asthma (Institute of Medicine, 2000, 2004). More recently, the World Health Organization found sufficient evidence to support associations between indoor dampness and both asthma development and exacerbation of existing asthma, recommending reductions in persistent dampness to reduce microbial growth (World Health Organization, 2009). Many previous studies examining the relationship between mold and asthma in children have relied on self-reported indicators of airborne fungi exposure such as visual assessment of mold or dampness (Antova et al., 2008; Cho et al., 2006; Mommers

et al., 2005; Simoni et al., 2005). The lack of objective measurement and exposure specificity in these studies also makes it difficult to identify the agents associated with respiratory diseases (Bornehag et al., 2001, 2004). Other studies have examined these relationships using either total mold or genera-specific concentrations from air samples as indicators of exposure, but have not assessed current asthma, instead relying on symptoms only or self-reported history of diagnosis (Rosenbaum et al., 2009; Su et al., 2001).

The aim of this study was to examine the relationship between indoor mold and risk of asthma in an urban population of children. Secondary objectives included identifying home environmental correlates of quantified fungal spores, specifically indoor relative humidity (RH) levels, and to examine genus-specific relationships with asthma.

Methods

Study design and population

This nested case-control study was conducted within a cross-sectional survey population of children up to 17 years of age living in Buffalo, New York. In the cross-sectional study, parents completed a questionnaire from which the child's respiratory health was assessed and data on numerous indoor environmental risk factors were collected. More detailed information on the methods used in the cross-sectional survey and the socio-demographic distribution of the study population can be found in two previously published papers (Jones et al., 2008; Lin et al., 2008) and an Agency for Toxic Substances and Disease Registry report (Agency for Toxic Substances and Disease Registry, 2005). Only the methods relevant to the case-control study are described in this paper.

A random sample of the asthma cases and noncases from the cross-sectional study ($N = 573$) was invited to participate in the case-control study involving clinical and home environmental testing. Children participating in the cross-sectional survey were assigned asthma case status on the basis of cross-sectional questionnaire responses. Case criteria were adapted from several standardized questionnaires (Asher et al., 1995; Crain et al., 1994; Salome et al., 1987) and included (i) ever having a physician diagnosis of asthma, (ii) having current asthma, (iii) ever wheezing, (iv) wheezing or whistling most days or nights in the past year, (v) dry cough at night in the past year, (vi) exercise-related wheezing in the past year and (vii) ever visiting an emergency room or hospital because of asthma. Children were classified as cases if they met any two of these criteria. We used these broad inclusion criteria to identify all potential asthma cases. Control subjects met none of these criteria (i.e., had no asthma diagnosis and none of the symptoms) and were

frequency-matched to cases by gender, race and age (within 2 years).

During phone recruitment, a series of screening questions was asked of the parent or guardian to determine the child's eligibility to participate. Children were ineligible if they reported an asthma diagnosis prior to moving to their current residence, had lived at the current residence for fewer than 6 months, or reported long-term use of corticosteroids or antihistamines. Control subjects who reported an asthma diagnosis or a sibling's asthma diagnosis during the subsequent clinical interview were also excluded. The health interview for the child was conducted at the Erie County Medical Center Department of Family Medicine, of the State University of New York at Buffalo. At the beginning of the interview, a parent or guardian was asked a series of questions from the cross-sectional questionnaire to verify the child's asthma status. These included questions about the child's medical history, including a history of hay fever, eczema or sinusitis, allergies, medication use and asthma symptoms. Lung function and skin prick testing for allergens (including dust-mites, cat, dog, cockroach, mouse and rat, but not fungi) was conducted as part of the clinical interview for the nested case-control study; results are available in a published report (Agency for Toxic Substances and Disease Registry, 2005). However, we did not use lung function tests to classify or confirm children as asthmatic for the purpose of our analyses, to maintain our intentionally broad inclusion criteria.

Home environmental sampling and exposure assessment

A subgroup of case and control homes was eligible and recruited for home environmental sampling if they completed the health and environmental surveys from the cross-sectional study and the child was a participant in the health interview. Indoor air samples were collected by trained field staff in the room where occupants spent most of their waking time (living room or family room). Staff also conducted a face-to-face interview with participants and conducted walk-through assessments of the home to collect additional information on home and indoor environmental characteristics. These data included moisture sources, the presence of carpeting throughout the home, cleaning practices and characteristics of heating, cooling and cooking devices. Dry bulb temperature and RH were measured in each home using a sling psychrometer. To minimize seasonal variation in measurement of environmental factors, indoor air sampling was performed for a similar proportion of frequency-matched cases and controls during the same season or on each sampling trip.

The objective of the air sampling was to characterize the distribution of airborne mold spore exposures across a sample of homes, rather than assess problem

conditions in a single building. Two sampling methods – total counts and viable counts – were used to determine fungal levels in indoor air. The two methods provide different, but complementary, information about airborne fungal levels. Total counts enumerate all airborne fungal spores but many are not precisely identified, while viable counts are more specific, but only enumerate a selected fraction of the total airborne fungal flora (Buttner et al., 1997).

A spore-trap sampler (Burkard Personal Volumetric Sampler; Burkard Manufacturing Co., Rickmansworth, UK) with a sampling rate of 10 l per minute was used to collect total spore-count samples. A glass microscope slide containing two-sided, optically clear adhesive tape was used for each sample to capture airborne particles from the sampled air (Recer, 2004). Sampling times were 30–60 min, depending on season. Spores were categorized taxonomically and enumerated under phase-contrast microscopy by an experienced mycologist following established protocols for spore-trap samples (Recer, 2004; Syzdek and Haines, 1995). Total counts are reported as spores per cubic meter (spores/m³) of air sampled.

Samples for viable airborne fungi were collected using an Andersen N6 sampler with a flow rate calibrated to 28.3 l per minute. Samples were collected for 2–5 min (depending on season) in duplicate on malt-dextrose agar and incubated at room temperature for 5–10 days, at which time colonies were enumerated and isolates identified to the genus level by an experienced mycologist. Sub-cultures were further analysed to identify *Aspergillus* isolates to species. Viable counts were adjusted using the positive hole correction factor (Andersen, 1958) and reported as colony forming units per cubic meter (CFU/m³) of air sampled.

Up to six homes were sampled during each sampling event of 2–3 days duration. Total count samples were stored in slide boxes at room temperature. Viable count samples were stored on ice in coolers until they were returned to the laboratory. At least one set of outdoor samples was collected during each sampling event, and more were collected if a significant change in weather conditions occurred during the sampling event. Field blanks for viable samples (i.e., sampling media placed in the Andersen sampler and then removed without being exposed to sampled air) were included in each sampling event as a control for sample contamination.

For the purpose of analysis, exposure to molds was categorized by the total or viable mold spore count and the fungal groups identified. For bivariate comparisons, counts were dichotomized as above or below the 85th percentile of concentrations for all sampled homes. This cut-point was used to capture a level of exposure which might be etiologically relevant, on the basis of airborne concentrations significantly associated with respiratory endpoints in studies conducted by others in the north-eastern United States (e.g., Stark

et al., 2003). We also assessed concurrent exposure to *Aspergillus* by counting the number of species identified in viable samples.

Statistical methods

SAS (v. 9.0) software was used for all statistical analyses (SAS Institute, Inc, 2005). Asthma classification was confirmed between self-reported status and status as determined in the clinical interview by measuring the crude agreement proportion between two dichotomous variables for case status. Univariate and bivariate methods were used to conduct descriptive analyses and to identify confounders and effect modifiers. Fungal-count data were not normally distributed, so nonparametric Mann–Whitney or Kruskal–Wallis tests were used for all two-sample comparisons. Associations between case–control status and home environment variables were assessed with contingency tables and unconditional logistic regression. Confidence intervals for exposure odds ratios reflect significance at $P \leq 0.10$. The decision to use a 90% CI was determined prior to the case–control analysis with the goal of hypothesis generation. Potential confounding by indoor and outdoor characteristics including frequent neighborhood truck traffic, chemical odors and other factors was assessed but none of these variables remained significant in the multivariable models. Two of the matching factors, race and gender, did not meet criteria for confounding in our data, as they were not significantly associated with exposure. Multivariable analyses therefore controlled only for family history of asthma and residual differences in age.

Results

The total sample size for the case–control study after exclusions included 202 individuals; 88 cases and 114 controls. Ninety-nine households agreed to participate in the home environmental sampling, so the final analysis dataset included 50 children meeting the asthma case definition and 49 controls. In data not shown, 33 (67%) cases had been diagnosed with asthma by a physician. The mean age of the children was 11 years (ranged from 3 to 17 years).

Distribution of mold in study homes

Cladosporium was the most prevalent taxon identified in viable count samples, found in 98% of homes, followed by *Penicillium* (91%), sterile (i.e., nonsporulating) fungi (90%), *Aspergillus* (68%), yeasts (67%), *Walemia* (38%) and *Aureobasidium* (27%). Other identified viable fungi were present in fewer than 12% of study homes. In the total-count samples,

Cladosporium (87%), ascospores (87%), *Penicillium/Aspergillus* (73%) and basidiospores (67%) were the most frequently identified fungal groups. Twenty-seven total-count categories identified in 1–43% of homes, including *Ganoderma*, *Coprinus*, *Alternaria*, smuts, *Pithomyces*, and various types of pollen. Overall prevalence of fungi in outdoor samples was very similar to that of indoor samples, with the top five fungal groups including *Cladosporium* (found outside 100% of homes), followed by sterile fungi (96%), *Aspergillus* (74%), *Penicillium* (70%) and yeasts (70%) (data not shown).

Mean viable counts were highest for *Cladosporium* (250 CFU/m³), followed by sterile fungi (180 CFU/m³), *Penicillium* (130 CFU/m³), *Aspergillus* (50 CFU/m³) and yeasts (35 CFU/m³). Mean total spore counts were highest for basidiospores (500 spores/m³), *Cladosporium* (240 spores/m³), ascospores (190 spores/m³) and *Penicillium/Aspergillus* (130 spores/m³). The median concentrations of some of the most prevalent fungi (i.e., *Penicillium*, *Aspergillus*) were modestly higher among the cases (Table 1); however, there were no statistically significant differences in the distribution of viable or total mold spore counts between cases and controls (all $P > 0.10$, Wilcoxon–Mann–Whitney U test).

Relationship between fungal levels and asthma status

Multivariable regression analyses were conducted to examine case status with respect to exposure to the most prevalent fungi in homes (Table 2). Family history of asthma showed a strong multiplicative interaction with the presence of elevated viable *Aspergillus* on the risk of asthma; therefore, separate models were generated after stratification by family history. Among children without a family history of asthma,

Table 1 Viable and total mold counts and predominant mold categories in indoor air, cases ($N = 50$) vs. controls ($N = 49$)

Mold	Cases		Controls	
	Median	Range	Median	Range
Viable counts (CFU/m ³) ^a				
Total	501	23–3388	481	16–3437
<i>Cladosporium</i>	110	0–2834	124	0–1582
Sterile fungi ^b	70	0–2073	79	0–739
<i>Penicillium</i>	45	0–586	41	0–2475
<i>Aspergillus</i>	24	0–620	18	0–402
Yeasts	17	0–225	15	0–215
Total counts (spores/m ³)				
Total	274	0–7580	265	0–10,963
<i>Cladosporium</i>	40	0–1840	40	0–2191
Ascospores	39	0–353	33	0–5390
<i>Penicillium/Aspergillus</i>	33	0–733	20	0–1789
Basidiospores	10	0–5867	38	0–7154

^aCFU, Colony forming units.

^bNonsporulating colonies.

Table 2 Multivariable logistic regression analyses of asthma and indoor air mold levels at or above the 85th percentile ($N = 99$)

Mold	Crude		Adjusted	
	OR ^a	90% CI	OR ^b	90% CI
Viable counts				
Total	1.37	0.52–3.56	1.59	0.54–4.72
<i>Aspergillus</i>	2.81	1.00–7.90		
Without family history of asthma ^c			6.11	1.37–27.19
With family history of asthma			0.54	0.10–2.92
Yeasts	1.93	0.72–5.17	1.37	0.45–4.15
<i>Cladosporium</i>	1.37	0.52–3.56	1.19	0.39–3.60
Sterile	0.98	0.38–2.52	1.30	0.46–3.64
<i>Penicillium</i>	0.49	0.19–1.31	0.35	0.11–1.17
Total counts				
Total	0.49	0.19–1.31	0.59	0.19–1.84
<i>Cladosporium</i>	1.93	0.73–5.14	2.37	0.77–7.26
Ascospores	0.70	0.27–1.82	1.15	0.38–3.84
Basidiospores	0.70	0.27–1.82	0.94	0.31–2.83
<i>Penicillium/Aspergillus</i>	0.70	0.27–1.82	0.50	0.16–1.56

^aBold face OR (odds ratio) and CI (confidence interval) indicate significance at the $\alpha = 0.10$ level.

^bAdjusted for age and one or more family members with asthma.

^cThere was a strong interaction between an elevated level of *Aspergillus* and one or more family members with asthma. Therefore, separate models were generated for individuals with and without a family member with asthma.

asthma cases had a significantly higher exposure to viable *Aspergillus* than controls (OR: 6.11, 90% CI: 1.37–27.19). In comparison, no association was found between exposure to viable *Aspergillus* and case status among children who did have a family history of asthma. The adjusted OR for elevated viable *Cladosporium* levels was suggestive of a positive association, but was not statistically significant (OR: 1.19, 90% CI: 0.39–3.60). There were no clear patterns observed in the odds of exposure by case status from molds measured in total count samples (ORs ranged from 0.50 to 2.37).

There was an apparent linear relationship between the number of *Aspergillus* species identified in viable samples and case status in the crude analyses (Figure 1). Although not statistically significant, the odds of exposure to four or more viable *Aspergillus* species among cases were nearly three times higher than controls (OR: 3.60, 90% CI: 0.88–14.65). Similarly, the odds of exposure to 2–3 species of *Aspergillus* compared to 0–1 was greater among the cases than controls (OR: 1.52, 90% CI 0.70–3.32). Additionally, a test for trend was statistically significant ($P < 0.05$), confirming the positive association between an increasing number of viable *Aspergillus* species identified in indoor air and asthma status (data not shown).

Relationship between reported home conditions and measured mold and humidity

Most self-reported home environmental characteristics were not significantly associated with measured levels of viable airborne fungi (Table 3). However, mean viable

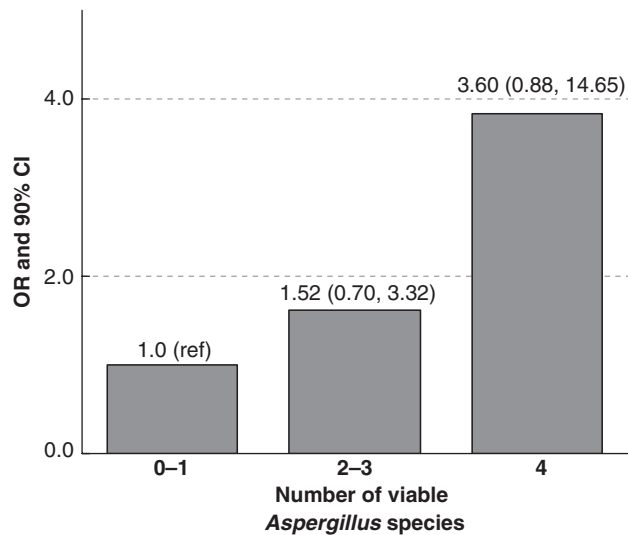


Fig. 1 Association between the number of viable *Aspergillus* species measured in the home and asthma status

fungal levels were significantly higher in homes where participants reported the presence of moldy odors (839 vs. 547 CFU/m³, $P = 0.0013$) and in homes with more than one bathroom (789 vs. 529 CFU/m³, $P = 0.0826$) (Table 3). Although nonsignificant, mean viable counts were also higher in homes reporting signs of mold growth (696 vs. 600 CFU/m³, respectively, $P = 0.2383$). Significantly higher mean total spore counts were also found in homes with reported signs of mold growth (1567 vs. 831 spores/m³), as well as having home furnishings professionally cleaned (1752 vs. 996 spores/m³) and in homes with moldy odors present (1574 vs. 996 spores/m³) (Table 3). Forty-nine of 99 homes (49.5%) reported some mold growth, 34 (34.3%)

reported moldy odors and 68 (69.4%) reported damp conditions, resulting from leaks in plumbing, roof, or the basement (data not shown). There were no significant differences in the report of these indicators between cases and controls.

The correspondence between RH in the living room of the home and viable and total fungal counts for the predominant fungal groups is presented in Table 4. Mean viable levels of *Cladosporium* (390 CFU/m³) and *Penicillium* (112 CFU/m³) were higher in homes with $\geq 50\%$ RH compared to those with $< 50\%$ RH (113 and 81 CFU/m³, respectively). Total spore count means for *Cladosporium*, ascospores, basidiospores and *Penicillium/Aspergillus* were also significantly higher in the homes with $\geq 50\%$ RH (ranged from 197 to 999 spores/m³) (Table 4).

Discussion

Using samples of airborne fungi, this study identified *Cladosporium*, *Penicillium* and *Aspergillus* species as some of the most frequently detected viable fungi in the homes of children living in Buffalo, New York. This is similar to results from other studies measuring indoor mold in the eastern United States (Gent et al., 2002; Horner et al., 2004; Shelton et al., 2002). *Cladosporium*, the most frequently identified mold in air samples, was positively but nonsignificantly associated with case status. Several other studies employing quantitative exposure assessments of indoor mold among child populations have also found a positive but indefinite relationship with respiratory endpoints. Stark et al. (2003) identified exposure to elevated levels of indoor airborne *Cladosporium* as a risk factor for lower respiratory illnesses in the first year of life in a

Table 3 Relationship between viable counts (CFU/m³), total spore counts (spores/m³) and home characteristics

Home characteristic	Viable counts mean (median)			Total counts mean (median)		
	Yes	No	P^a	Yes	No	P^a
Install new carpet	638 (497)	650 (481)	0.9409	736 (567)	1311 (265)	0.8548
Home furnishings professionally cleaned last year	747 (607)	612 (462)	0.2760	1752 (970)	996 (253)	0.0617
Painted/varnished home last year	695 (575)	580 (338)	0.2802	1347 (431)	979 (147)	0.1132
Use natural gas for cooking	655 (498)	582 (332)	0.9645	1269 (269)	605 (368)	0.8368
Use gas for heating	630 (480)	2331 (2331)	0.1075	1206 (274)	147 (147)	0.7003
Use fuel oil for heating	2331 (2331)	630 (480)	0.1075	147 (147)	1206 (274)	0.7003
Use propane for heating	752 (752)	646 (480)	0.5519	147 (147)	1206 (274)	0.7003
Use kerosene for heating	319 (319)	651 (498)	0.7528	99 (99)	1206 (274)	0.5061
Use of humidifier	656 (480)	637 (515)	0.7679	1253 (290)	1125 (254)	0.8358
Use of dehumidifier	614 (467)	660 (481)	0.6777	1080 (197)	1240 (314)	0.2665
Use of air conditioning	642 (541)	650 (376)	0.8278	814 (274)	1453 (253)	0.5659
Signs of mold growth	696 (592)	600 (335)	0.2383	1567 (530)	831 (165)	0.0343
Moldy odors	839 (753)	547 (308)	0.0013	1574 (654)	996 (213)	0.0311
>1 bathroom	789 (592)	529 (335)	0.0826	1140 (530)	1241 (199)	0.6055
Workshop	709 (524)	603 (471)	0.4930	995 (314)	1336 (269)	0.8285
Smoker in home	596 (405)	664 (515)	0.9967	787 (218)	1325 (306)	0.4428
Stove fan	518 (419)	712 (575)	0.2138	1025 (500)	1280 (259)	0.7637
Stove vent to outside	613 (340)	653 (523)	0.5011	1044 (612)	1220 (254)	0.5739

^aKruskal–Wallis test. Bold face indicates significance at the $\alpha = 0.10$ level.

Table 4 Comparison of mean indoor viable and total fungi measured in homes with <50% humidity vs. homes with ≥50% humidity^a

	Mean (median)		
	≥50% Humidity	<50% Humidity	<i>P</i> -value ^b
Viable counts (CFU/m ³)			
Total	838 (742)	420 (256)	0.0005
<i>Alternaria</i>	0.7 (0)	1 (0)	0.4826
<i>Aspergillus</i>	53 (24)	49 (16)	0.4597
<i>Aureobasidium</i>	5 (0)	6 (0)	0.2534
<i>Cladosporium</i>	390 (205)	113 (55)	0.0002
<i>Epicoccum</i>	0.5 (0.0)	2 (0)	0.5210
<i>Mucor</i>	0.3 (0.0)	2 (0)	0.9117
<i>Penicillium</i>	112 (61)	81 (36)	0.0298
Sterile	228 (95)	147 (49)	0.6273
<i>Walemia</i>	40 (0)	13 (0)	0.8382
Yeast	35 (17)	36 (17)	0.7852
Others	4 (0)	2 (0)	0.3176
Total counts (spores/m ³)			
Total	2051 (1155)	376 (72)	<0.0001
<i>Cladosporium</i>	313 (128)	198 (15)	0.0008
Ascospores	336 (96)	38 (15)	<0.0001
Basidiospores	999 (162)	21 (0)	<0.0001
<i>Penicillium/Aspergillus</i>	197 (53)	23 (15)	0.0007

^aMeasured in the living room of the home.^bKruskal–Wallis test. Bold face indicates significance at the $\alpha = 0.10$ level.

prospectively evaluated Massachusetts birth cohort (RR: 1.17). Rosenbaum et al. (2009) also utilized air sampling and genus-specific measures of indoor mold exposure and prospectively evaluated risk of respiratory symptoms among infants in upstate New York. Similar to our study, positive but nonstatistically significant associations were identified between high levels of *Cladosporium* and wheeze (OR: 2.28). A case–control study conducted in Australia also found an association between airborne *Cladosporium* and respiratory symptoms among children aged 7–14 years after adjustment for parental allergy and asthma (Garrett et al., 1998). Therefore, our finding that *Cladosporium* exposures were greater (although effect estimates are imprecise) among the asthma cases in our population is largely consistent with other studies employing similar sampling methodology and quantitative exposure assessments, several of which were also conducted in the north-eastern United States. We did not identify a strong positive association between airborne *Penicillium* and asthma, as been found by others (Garrett et al., 1998; Gent et al., 2002; Rosenbaum et al., 2009; Stark et al., 2003). However, direct comparisons of the findings may be limited by imprecise effect estimates and potential differences in mold sensitivity as a result of the higher average age of the children in our study population (11 years vs. 1 year of age in Gent et al., Rosenbaum et al. and Stark et al.; ages ranged from 7 to 14 years with no mean reported in Garrett et al.), and potentially also by design differences.

A major finding from this study was the sixfold increased odds of exposure to *Aspergillus* among

asthmatic children who had no family history of asthma, a well-known risk factor for the disease (Arruda et al., 2005). One potential explanation for the lack of association among those with a family history is that atopic sensitization may not play a role in asthma triggered by fungal exposure, as was observed in an adult population (Cox-Ganser et al., 2005). Another possible reason for the lack of association could be that knowledge of an immediate family member's status as asthmatic leads to greater avoidance of triggers, which is a cornerstone of asthma management in children (Diette et al., 2008; Huss et al., 2003; Kemp and Kemp, 2001). Several studies of such trigger avoidance among children with family history or atopic parents have also described a reduction in risk of respiratory impairments (Arshad, 2004; Custovic et al., 2001; Woodcock et al., 2004), demonstrating the biologic plausibility of our finding. Notably, this interaction with family history was not observed among exposures to other fungal genera, which may imply that genus- or species-specific fungal exposure is important in asthma risk independent of family history. Although this result suggests that particular species may have greater potency as asthma triggers, it is unlikely that trigger reduction efforts aimed at specific fungal species would be successful (absent a known source of overt indoor fungal contamination). More general trigger reduction efforts aimed at reducing indoor dampness would be expected to benefit all individuals at increased risk for asthma symptoms from fungal exposures. Finally, the lack of observed association may also be explained by limited data.

Another important finding was the almost threefold increase in asthma risk among children with four or more species of *Aspergillus* identified from viable samples. Because total mold levels were not significantly associated with case status, this result suggests a potential genus-specific relationship with asthma. *Aspergillus* species are known allergens (Mari et al., 2003) which contribute to a variety of respiratory disease processes, including asthma (Katayama et al., 2008; Vesper et al., 2008). The results also suggest that concurrent exposure to multiple species of fungal genera might play a role in asthma risk. However, although nineteen different *Aspergillus* species were identified in study homes, we were unable to assess whether concurrent exposure to multiple species of other important allergenic fungi (e.g., *Cladosporium* or *Penicillium*) demonstrated similar associations with asthma risk, because isolates of these genera were not speciated. Similarly, the lack of any significant associations with total spore counts may be due in part to the lack of precise species identification in relevant total-count samples. Therefore, our finding, while suggestive, warrants additional exploration.

We also found that self-reported moisture-related home characteristics, including moldy odors and

having more than one bathroom in the home, were associated with total indoor fungi levels. This association was independent of case status and is consistent with well-known relationships between indoor mold growth and available moisture sources. Other studies have found that visual identification of mold and moisture sources corresponded with elevated levels of indoor fungi (Dales et al., 1997; Garrett et al., 1998). Therefore, our finding is important in the context of the body of evidence in support of associations between dampness, mold and respiratory symptoms (Fisk et al., 2007). Although moldy odors was the most sensitive among self-reported measures, we did not observe associations between total mold levels and all conditions which might signify mold, indicating that not all subjective indicators of dampness can serve as surrogates for the presence of indoor fungi. This was demonstrated in a study by Rosenbaum et al. (2009), which found that airborne molds were significantly associated with wheeze but that visual indicators of mold and dampness were not, and with findings by Ren et al. (2001) and Denning et al. (2006), which suggest that home characteristics and visual assessments of dampness cannot reliably predict the presence of indoor mold.

High RH levels measured in the home were associated with both average viable and total-count samples of indoor fungi and several specific genera, including *Cladosporium* and *Penicillium*, which are known allergenic molds (Jacob et al., 2002). Similar associations between humidity and indoor fungal levels were reported from a multi-city survey of asthmatic children in urban areas across the United States, which found that RH was positively associated with high indoor airborne fungal concentrations and with elevated levels of specific fungi (O'Connor et al., 2004). The lack of a consistent association between high RH and some of the fungi identified in our study (e.g., *Aspergillus*) could be due to variations in the determinants of growth related to humidity or the cross-sectional nature of the humidity measure, which may not reflect the necessary conditions for mold growth.

Strengths and Limitations

Strengths of this study include that we collected detailed information on respiratory health history and indoor environmental risk factors and used sampling data to characterize indoor mold exposure in a susceptible inner-city US population. Our quantitative assessment helped to avoid differential misclassification of exposure, as cases may be more likely to report visible fungal growth because of heightened awareness of mold as an asthma trigger (Dales et al., 1997; Strachan et al., 1990). This is also one of the few studies of mold exposure in children

to quantify both viable and total spores. Moreover, the sampling allowed us to identify levels of mold that would not be discernable by visual inspection, which might represent lower levels of mold only pertinent to asthma risk among highly sensitive individuals.

The potential for bias in the self-reported health information was addressed by asking few open-ended questions and specifying frequency for symptoms. To prevent disease misclassification because of a lack of information regarding the child's current asthma management, we defined cases based on and current symptoms, using details from both the clinical and cross-sectional surveys. Data were collected only for the eldest child in the home, which minimized a potential overemphasis of family history of asthma and shared indoor environmental exposures within a single household.

Despite these strengths, there remain potential limitations to the study findings. First, small sample sizes for the analysis because of the large number of fungal groups examined resulted in poor precision for some estimates. Second, it is likely that children who live in homes with mold are also exposed to other indoor environmental risk factors for asthma and that some relevant exposures may occur away from home, such as at school. To address this concern, we examined multiple self-reported indoor and outdoor environmental factors as potential confounders. Other explanatory variables for asthma status were used as matching variables to improve efficiency in control of confounding by these factors. Another limitation is that mold allergen sensitization and cross-reactivity were not evaluated for these analyses, which could serve to modify asthma risk following mold exposure. Therefore, although we cannot discount the possibility of influences by unmeasured factors, including a potential relationship with other allergens, we made attempts to control for some known risk factors in our multivariable analyses.

Another potential limitation arises from the nature of our sampling activity. We collected at least one outdoor sample on each sampling trip, from which concentrations were consistently correlated with indoor concentrations of fungi. This reflects the expected intrusion of mold spores in outdoor air into the home. Although adjusting for any indoor-outdoor difference may be appropriate, as has been done by others (O'Connor et al., 2004), outdoor mold counts can be affected by numerous factors, including wind, temperature, humidity and precipitation, rendering them unreliable as a cross-sectional indicator of exposure. Additionally, our most suggestive findings were for *Aspergillus*, which tends to be detected more frequently in indoor air samples compared to concomitant outdoor samples in air sampling surveys (e.g., Recer, 2004 and references cited therein). For these reasons, we

suggest that the use of the unadjusted sampled concentrations in our analysis was appropriate. We additionally acknowledge that grab samples for viable and total fungal spores are subject to significant spatial and temporal variability, limiting the utility of a small number of samples to assess the presence of mold amplification in a single home. Rather than assess problem conditions in a single building, as might be conducted with remediation efforts in mind, our study attempted to characterize the distribution of airborne mold spore exposures across a sample of homes.

Finally, the case sampling method used for this study is subject to potential selection bias. We identified a random subset of our cross-sectional population for recruitment. To allay concerns about possible selection effects, including the possibility that cases might be more willing to participate, we made several comparisons via chi-squared tests. First, we compared the participation rates between invited cases and controls and found no statistically significant differences. We then compared demographic characteristics between participants and nonparticipants and found only non-significant differences in racial composition. Thus, we are reasonably certain that our case-control popula-

tion was representative of our source population and was not influenced by obvious selection biases.

Conclusion

This study found mold levels and fungal groups identified in case and control homes to be consistent with similar studies conducted in the north-eastern United States. The observed rate of exposure to mold and specifically to some allergenic molds was generally higher among the cases. An interaction between family history and fungal exposure was identified and may reflect trigger avoidance in individuals with known susceptibility to asthma. Measured humidity levels were positively associated with several fungi and most strongly with the total count data. Finally, some self-reported indicators of mold were better predictors of the presence of indoor fungi than others, with moldy odors emerging as the most sensitive of these indices. These findings generally confirm known associations between indoor mold exposures and asthma status and warrant future investigation to determine the utility of visual or other assessments of mold exposure in lieu of indoor air sampling.

References

- Agency for Toxic Substances and Disease Registry (2005) Childhood asthma and environmental risk factors in the city of buffalo, New York. PB2005-102180, 1-137.
- Akinbami, L.J. and Schoendorf, K.C. (2002) Trends in childhood asthma: prevalence, health care utilization, and mortality, *Pediatrics*, **110**, 315-322.
- Akinbami, L.J., Moorman, J.E., Garbe, P.L. and Sondik, E.J. (2009) Status of childhood asthma in the United States, 1980-2007, *Pediatrics*, **123**, S131-S145.
- Andersen, A.A. (1958) New sampler for the collection, sizing and enumeration of viable airborne particles, *J. Bacteriol.*, **76**, 471-484.
- Antova, T., Pattenden, S., Brunekreef, B., Heinrich, J., Rudnai, P., Forastiere, F., Luttmann-Gibson, H., Grize, L., Katsnelson, B., Moshhammer, H., Nikiforov, B., Slachtova, H., Slotova, K., Zlotkowska, R. and Fletcher, T. (2008) Exposure to indoor mould and children's respiratory health in the PATY study, *J. Epidemiol. Community Health*, **62**, 708-714.
- Arruda, L.K., Sole, D., Baena-Cagnani, C.E. and Naspitz, C.K. (2005) Risk factors for asthma and atopy, *Curr Opin Allergy Clin Immunol*, **5**, 153-159.
- Arshad, S.H. (2004) Allergen avoidance and prevention of atopy, *Curr Opin Allergy Clin Immunol*, **4**, 119-123.
- Asher, M.I., Keil, U., Anderson, H.R., Beasley, R., Crane, J., Martinez, F., Mitchell, E.A., Pearce, N., Sibbald, B., Stewart, A.W., Strachan, D., Weiland, S.K. and Williams, H.C. (1995) International Study of Asthma and Allergies in Childhood (ISAAC): rationale and methods, *Eur. Respir. J.*, **8**, 483-491.
- Belanger, K., Beckett, W., Triche, E., Bracken, M.B., Holford, T., Ren, P., McSharry, J.E., Gold, D.R., Platts-Mills, T.A. and Leaderer, B.P. (2003) 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.*, **158**, 195-202.
- Bornehag, C.G., Blomquist, G., Gyntelberg, F., Jarvholm, B., Malmberg, P., Nordvall, L., Nielsen, A., Pershagen, G. and Sundell, J. (2001) Dampness in buildings and health. Nordic interdisciplinary review of the scientific evidence on associations between exposure to "dampness" in buildings and health effects (NORD-DAMP), *Indoor Air*, **11**, 72-86.
- Bornehag, C.G., Sundell, J. and Sigsgaard, T. (2004) Dampness in buildings and health (DBH): report from an ongoing epidemiological investigation on the association between indoor environmental factors and health effects among children in Sweden, *Indoor Air*, **14**(Suppl 7), 59-66.
- Buttner, M.P., Willeke, K. and Grinshun, S.A. (1997) Sampling and analysis of airborne microorganisms. In: Hurst, C.J., Knudsen, G.R., McInerney, M.J., Stetzenbach, L.D. and Walter, M.V. (eds) *Manual of Environmental Microbiology*, ASM Press, Washington DC, 629-640.
- Cho, S.H., Reponen, T., LeMasters, G., Levin, L., Huang, J., Meklin, T., Ryan, P., Villareal, M. and Bernstein, D. (2006) Mold damage in homes and wheezing in infants, *Ann. Allergy Asthma Immunol.*, **97**, 539-545.
- Cox-Ganser, J.M., White, S.K., Jones, R., Hilsbos, K., Storey, E., Enright, P.L., Rao, C.Y. and Kreiss, K. (2005) Respiratory morbidity in office workers in a water-damaged building, *Environ. Health Perspect.*, **113**, 485-490.
- Crain, E.F., Weiss, K.B., Bijur, P.E., Hersh, M., Westbrook, L. and Stein, R.E. (1994) An estimate of the prevalence of asthma and wheezing among inner-city children, *Pediatrics*, **94**, 356-362.
- Custovic, A., Simpson, B.M., Simpson, A., Kissen, P. and Woodcock, A. (2001) Effect of environmental manipulation in pregnancy and early life on respiratory symptoms and atopy during first year of life: a randomised trial, *Lancet*, **358**, 188-193.
- Dales, R.E., Miller, D. and McMullen, E. (1997) Indoor air quality and health: validity and determinants of reported home dampness and moulds, *Int. J. Epidemiol.*, **26**, 120-125.
- Denning, D.W., O'Driscoll, B.R., Hoga-boam, C.M., Bowyer, P. and Niven, R.M.

- (2006) The link between fungi and severe asthma: a summary of the evidence, *Eur. Respir. J.*, **27**, 615–626.
- Diette, G.B., McCormack, M.C., Hansel, N.N., Breyse, P.N. and Matsui, E.C. (2008) Environmental issues in managing asthma, *Respir Care*, **53**, 602–615.
- Fisk, W.J., Lei-Gomez, Q. and Mendell, M.J. (2007) Meta-analyses of the associations of respiratory health effects with dampness and mold in homes, *Indoor Air*, **17**, 284–296.
- Garrett, M.H., Rayment, P.R., Hooper, M.A., Abramson, M.J. and Hooper, B.M. (1998) Indoor airborne fungal spores, house dampness and associations with environmental factors and respiratory health in children, *Clin. Exp. Allergy*, **28**, 459–467.
- Gent, J.F., Ren, P., Belanger, K., Triche, E., Bracken, M.B., Holford, T.R. and Leaderer, B.P. (2002) Levels of household mold associated with respiratory symptoms in the first year of life in a cohort at risk for asthma, *Environ. Health Perspect.*, **110**, A781–A786.
- Heyman, K.M., Schiler, J.S. and Barnes, P.M. (2008) Early release of selected estimates based on data from the January-June 2008 National Health Interview Survey, National Center for Health Statistics, Hyattsville, MD.
- Horner, W., Worthan, A.G. and Morey, P.R. (2004) Air- and dustborne mycoflora in houses free of water damage and fungal growth, *Appl. Environ. Microbiol.*, **70**, 6394–6400.
- Huss, K., Travis, P. and Huss, R.W. (2003) General principles of asthma management: environmental control, *Nurs. Clin. North Am.*, **38**, 609–620.
- Institute of Medicine (2000) *Clearing the Air: Asthma and Indoor Exposures*. National Academy of Sciences, Washington, D.C., The National Academies Press.
- Institute of Medicine (2004) *Damp Indoor Spaces and Health*. National Academy of Sciences, Washington, D.C., The National Academies Press.
- Jacob, B., Ritz, B., Gehring, U., Koch, A., Bischof, W., Wichmann, H.E. and Heinrich, J. (2002) Indoor exposure to molds and allergic sensitization, *Environ. Health Perspect.*, **110**, 647–653.
- Jones, R., Lin, S., Munsie, J.P., Radigan, M. and Hwang, S.A. (2008) Racial/ethnic differences in asthma-related emergency department visits and hospitalizations among children with wheeze in Buffalo, New York, *J. Asthma*, **45**, 916–922.
- Katayama, N., Fujimura, M., Yasui, M., Ogawa, H. and Nakao, S. (2008) Hypersensitivity pneumonitis and bronchial asthma attacks caused by environmental fungi, *Allergol Int*, **57**, 277–280.
- Kemp, J. and Kemp, J.A. (2001) Management of asthma in children, *Am. Fam. Physician*, **63**, 1341–1348.
- Lin, S., Gomez, M.I., Hwang, S.A., Munsie, J.P. and Fitzgerald, E.F. (2008) Self-reported home environmental risk factors for childhood asthma: a cross-sectional study of children in Buffalo, New York, *J. Asthma*, **45**, 325–332.
- Mari, A., Schneider, P., Wally, V., Breitenbach, M. and Simon-Nobbe, B. (2003) Sensitization to fungi: epidemiology, comparative skin tests, and IgE reactivity of fungal extracts, *Clin. Exp. Allergy*, **33**, 1429–1438.
- Mommers, M., Jongmans-Liedekerken, A.W., Derkx, R., Dott, W., Mertens, P., van Schayck, C.P., Steup, A., Swaen, G.M., Ziemer, B. and Weishoff-Houben, M. (2005) Indoor environment and respiratory symptoms in children living in the Dutch-German borderland, *Int J Hyg Environ Health*, **208**, 373–381.
- National Center for Health Statistics. (2006) *Health, United States, 2006*. With Chartbook on Trends in the Health of Americans, U.S. Department of Health and Human Services, Hyattsville, MD.
- O'Connor, G.T., Walter, M., Mitchell, H., Kattan, M., Morgan, W.J., Gruchalla, R.S., Pongracic, J.A., Smartt, E., Stout, J.W., Evans, R., Crain, E.F. and Burge, H.A. (2004) Airborne fungi in the homes of children with asthma in low-income urban communities: the Inner-City Asthma Study, *J. Allergy Clin. Immunol.*, **114**, 599–606.
- Pearce, N. and Douwes, J. (2005) Commentary: asthma time trends—mission accomplished?, *Int. J. Epidemiol.*, **34**, 1018–1019.
- Recer, G.M. (2004) A review of the effects of impermeable bedding encasements on dust-mite allergen exposure and bronchial hyper-responsiveness in dust-mite-sensitized patients, *Clin. Exp. Allergy*, **34**, 268–275.
- Ren, P., Jankun, T.M., Belanger, K., Bracken, M.B. and Leaderer, B.P. (2001) The relation between fungal propagules in indoor air and home characteristics, *Allergy*, **56**, 419–424.
- Rosenbaum, P.F., Crawford, J.A., Anagnost, S.E., Wang, C.J., Hunt, A., Anbar, R.D., Hargrave, T.M., Hall, E.G., Liu, C.C. and Abraham, J.L. (2009) Indoor airborne fungi and wheeze in the first year of life among a cohort of infants at risk for asthma, *J. Expo Sci Environ Epidemiol*, **20**, 503–515.
- Salome, C.M., Peat, J.K., Britton, W.J. and Woolcock, A.J. (1987) Bronchial hyper-responsiveness in two populations of Australian schoolchildren. I. Relation to respiratory symptoms and diagnosed asthma, *Clin. Allergy*, **17**, 271–281.
- SAS Institute, Inc. (2005) *Statistical Application System (SAS) Software, version 9.0*. Cary, NC.
- Shelton, B.G., Kirkland, K.H., Flanders, W.D. and Morris, G.K. (2002) Profiles of airborne fungi in buildings and outdoor environments in the United States, *Appl. Environ. Microbiol.*, **68**, 1743–1753.
- Silverstein, M.D., Mair, J.E., Katusic, S.K., Wollan, P.C., O'Connell, E.J. and Yunginger, J.W. (2001) School attendance and school performance: a population-based study of children with asthma, *J. Pediatr.*, **139**, 278–283.
- Simoni, M., Lombardi, E., Berti, G., Rusconi, F., La, G.S., Piffer, S., Petronio, M.G., Galassi, C., Forastiere, F. and Viegi, G. (2005) Mould/dampness exposure at home is associated with respiratory disorders in Italian children and adolescents: the SIDRIA-2 Study, *Occup. Environ. Med.*, **62**, 616–622.
- Stark, P.C., Burge, H.A., Ryan, L.M., Milton, D.K. and Gold, D.R. (2003) Fungal levels in the home and lower respiratory tract illnesses in the first year of life, *Am. J. Respir. Crit. Care Med.*, **168**, 232–237.
- Strachan, D.P., Flannigan, B., McCabe, E.M. and McGarry, F. (1990) Quantification of airborne moulds in the homes of children with and without wheeze, *Thorax*, **45**, 382–387.
- Su, H.J., Wu, P.C., Chen, H.L., Lee, F.C. and Lin, L.L. (2001) Exposure assessment of indoor allergens, endotoxin, and airborne fungi for homes in southern Taiwan, *Environ. Res.*, **85**, 135–144.
- Syzdek, L.D. and Haines, J.H. (1995) Monitoring *Aspergillus fumigatus* aerosols from a composting facility, *Aerobiologia*, **11**, 87–93.
- Vesper, S., McKinstry, C., Haugland, R., Neas, L., Hudgens, E., Heidenfelder, B. and Gallagher, J. (2008) Higher Environmental Relative Moldiness Index (ERMIsm) values measured in Detroit homes of severely asthmatic children, *Sci. Total Environ.*, **394**, 192–196.
- Wever-Hess, J., Kouwenberg, J.M., Duiverman, E.J., Hermans, J. and Wever, A.M. (2000) Risk factors for exacerbations and hospital admissions in asthma of early childhood, *Pediatr. Pulmonol.*, **29**, 250–256.
- Woodcock, A., Lowe, L.A., Murray, C.S., Simpson, B.M., Pipis, S.D., Kissen, P., Simpson, A. and Custovic, A., NAC Manchester Asthma and Allergy Study Group. (2004) Early life environmental control: effect on symptoms, sensitization, and lung function at age 3 years, *Am. J. Respir. Crit. Care Med.*, **170**, 433–439.
- World Health Organization (2009). *WHO Guidelines on Indoor Air Quality: Dampness and Mould*. Copenhagen, WHO Regional Office for Europe. (http://www.euro.who.int/InformationSources/Publications/Catalogue/20090629_4 accessed May 11, 2010)