Indoor Exposure to Molds and Allergic Sensitization

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Evidence that indoor dampness and mold growth are associated with respiratory health has been accumulating, but few studies have been able to examine health risks in relation to measured levels of indoor mold exposure. In particular, little is known about the contribution of indoor molds to the development of allergic sensitization. As a part of an ongoing study examining the effects of ambient air pollutants on respiratory health and atopic diseases in German school children, we examined the relation between viable mold levels indoors and allergic sensitization in 272 children. We examined whether allergic sensitization in children is associated with higher fungal spore count in settled house dust sampled from living room floors. Adjusting for age, sex, parental education, region of residency, and parental history of atopy, we found that mold spore counts for *Cladosporium* and *Aspergillus* were associated with an increased risk of allergic sensitization. Sensitized children exposed to high levels of mold spores (> 90th percentile) were more likely to suffer from symptoms of rhinoconjunctivitis. We conclude that elevated indoor concentrations of molds in wintertime might play a role in increasing the risk of developing atopic symptoms and allergic sensitization not only to molds but also to other common, inhaled allergens. These effects were strongest in the group of children who had lived in the same home since birth. *Key words:* allergic sensitization, house dust, indoor allergen exposure, molds. *Environ Health Perspect* 110:647–653 (2002). [Online 24 May 2002]
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The development of allergic sensitization and atopic disease in children is a function of a genetic predisposition to react to antigens and the timing and extent of exposure to allergenic agents. Evidence is accumulating that indoor allergen exposure early in life stimulates the development of allergic sensitization (*1–3*). Because most children and adults spend most of their time indoors, much attention has been directed to identifying indoor sources of allergens. House dust is a complex mixture of various biocontaminants and a major source of allergens in nonindustrial indoor environments. House dust contains allergens such as mites, epithels of pet dander, and molds (*1–6*), and threshold values at which exposure may cause sensitization have been proposed. Although some authors suggest sensitization to molds as a risk factor for allergic diseases and asthma (*7,8*), it is still unclear whether allergic sensitization is a risk factor for asthma (*9*). Epidemiologic studies reported positive associations between respiratory symptoms and living in damp houses (*10–16*), a condition thought to permit mold growth. Yet little is known about the contribution of indoor mold levels to allergic sensitization rates (*17,18*). Some reasons for the lack of information are that environmental monitoring is time and cost intensive and requires a high level of subject cooperation. In the present study we were able to use actual measurement of mold spores instead of relying on self-reports of mold growth to examine the role viable mold spores contained in house dust play in causing allergic sensitization and asthmatic and allergic symptoms in children.

Materials and Methods

Study population and selection of homes. We conducted two cross-sectional surveys in 1992–1993 and 1995–1996 to study the long-term health effects of ambient air pollution in German school children ages 5–14 years living in three areas of Saxony-Anhalt [n = 2,470 children (89.1%) and n = 2,814 (74.7%) participation rate, respectively, for each survey]. In both surveys, we elicited information about social and environmental factors (*19*) and asked parents to report allergic and respiratory symptoms and diseases for their children. In addition, our study physician examined all children and drew blood samples. For a select subgroup of children we were able to collect additional data, including samples of house dust and information concerning building and housing characteristics and living habits.

Drawing from both survey populations, we selected affected (case) and unaffected (control) children; cases were defined as children who could be classified as atopic according to at least one of the following three criteria: a positive skin prick test; at least one positive specific IgE test (CAP-RAST-FEIA; IgE > 0.35 kU/L); or physician diagnosis of asthma at any time before the survey. We used a stratified random sampling approach to select children in two age groups (5–7 and 8–10 years) from three residential areas; 80 children each were selected in the younger age group and between 40 and 50 in the older age group for a total of 370 case and 370 control children. Parents of 231 selected case children (62%) agreed to participate. Control children had to be nonatopic and nonasthmatic (i.e., they did not meet any of the above-mentioned criteria for cases. Parents of 223 selected control children (60%) agreed to participate. Overall, parents of 454 children allowed us to collect household dust samples.

Trained personnel performed interviews to document housing characteristics and visited homes twice at an interval of approximately 6 months to collect two dust samples. All 454 homes were visited between 1996 and 1998, but for the following analyses we considered only the homes of 340 children (178 case and 162 control children) who did not move between the medical examination and the home visit. For the following secondary analyses focusing on mold exposure and allergic sensitization, we excluded 20 subjects with missing data for IgE sensitization and seven subjects missing other covariate data. We further restricted our case group to sensitized children only (i.e., those children testing positive in at least one RAST test), excluding 41 children who qualified as

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cases only according to a positive skin-prick test. We considered the validity and reliability of the prick test results questionable because different test kits were used in each survey. Thus, 115 cases and 157 controls remained in the analyses. We chose to use only house dust samples taken in winter (November–April) to minimize the influence of seasonal variation (*20*) and to make our results comparable to previous studies using a similar restriction to winter sampling (*8,10,11,21–24*).

Approval of the study protocol was granted by the Ethics Committees of the University of Rostock and the University of Munich (LMU), and the study was performed in accordance with the institutional guidelines for the protection of human subjects. Written informed consent was obtained from the parents of all participating children.

House dust sampling and mold identification. Dust sampling and extraction procedures were identical to those used in a parallel study of adults (*25*) and described in more detail elsewhere (*20,26*). In each home, a dust sample was taken from the living room floor (97% were carpeted floors) by vacuuming an area of 1 m^2 for 2 min in a highly standardized manner using the same type of vacuum cleaner (Type Flüsterjet Vitall 371, 1,000 W; Phillips, Hamburg, Germany) and the same device (collector and filter; ALK, Hørsholm, Denmark) to collect dust on a paper filter (*20*). In general, samples were obtained from carpets. The dust filters were weighed before and after vacuuming to analyze the settled dust gravimetrically. The dust samples were stored at room temperature and analyses were performed within 10 days after sampling.

We analyzed 30 mg (500 µm) sieved house dust for identification and quantification of viable molds. Dusts were diluted in 0.9% NaCl and plated on DG18 (dichloran-18% glycerol agar) and 0.1 g/L chloramphenicol was added to prevent bacterial growth. Plates were incubated at 25°C for 10 days (*20,27*), and all analyses were duplicated. The number of colony-forming units (CFU) was counted and expressed as CFU per gram of dust. Colonies were identified to genus level using high-powered microscopy (Ergaval; Carl Zeiss, Jena, Germany).

The total number of CFUs may be of limited clinical and epidemiologic relevance because spores from different species have different allergenic potential (*11*). Therefore, we studied both the total counts and the counts of selected mold genera separately. The detection limit for total molds was 1,000 CFU/g dust, and, in some cases (high concentrations of total molds) for genus-specific CFU, 10,000 CFU/g dust.

Allergic sensitization. Blood collection, centrifugation of blood, and serum storage followed the protocol of the European Community Respiratory Health Survey (ECRHS) (*19,28*). The serum samples were stored at –20°C. Specific IgE for *Dermatophagoides pteronyssinus* (d1), cat allergens (e1), *Cladosporium* (m2), mixed grasses (g6), and birch (t3) were measured by the CAP-FEIA method by Pharmacia Diagnostics (Freiburg, Germany) using identical batches of reagents for all assays (*29*). The measurement range was 0.35–100 kU/L, with a detection limit < 0.35 kU/L. Allergic sensitization was defined as testing positive for at least one specific IgE (≥ 0.35 kU/L).

Statistical analysis. We performed statistical analyses using the statistical analysis package SAS for Windows version 6.12 (SAS Institute, Cary, NC, USA). We included in our multivariate analyses only those children for whom we had complete covariate data for potential risk factors for atopic diseases (age, sex, region of residency, educational level of the parents, and positive parental history of atopy) in addition to outcome and exposure information. Thus, we performed a complete-subject analysis for 272 children (115 sensitized cases and 157 controls).

Because of the log-normal distribution of mold spore counts, we present the median and the 25th and 90th percentile as measures of variation. We calculated the crude prevalence for sensitized cases and controls for binary response variables of allergic diseases or symptoms. We used the nonparametric Spearman rank-order coefficient (r_s) to determine the relationships between the CFU of several genera of mold spores.

Because established thresholds for mold genera are lacking, we classified subjects into three exposure categories (subjects exposed ≤ 25th, 25th–90th, and > 90th percentile). Multiple logistic regression analyses allowed us to examine the effect of mold spore exposures on allergic sensitization, atopic symptoms, and atopic diseases adjusting for a fixed set of potential confounding variables (age, sex, region of residency, educational level of the parents, and positive parental history of atopy) by including them in the model. We report adjusted odds ratios (OR) and 95% confidence intervals (CI) for each allergic outcome and mold exposure category.

Table 1. Basic description of the study population of 272 children in eastern Germany.

Characteristics	Sensitized cases (*n* = 115)		Controls (*n* = 157)	
	No.	Percent	No.	Percent
Demographic characteristic				
Place of residency				
Zerbst	42	36.5	49	31.2
Bitterfeld	20	17.4	32	20.4
Hettstedt	53	46.1	76	48.4
Sex				
Boys	71	61.7	79	50.3
Girls	44	38.3	78	49.7
Age				
5–7 years	69	60.0	94	59.9
8–10 years	46	40.0	63	40.1
Parental education				
≤ 10 grades	58	50.4	83	52.9
≥ 12 grades	57	49.6	74	47.1
Positive family history of atopy	29	25.2	27	17.2
Dampness at home	29	25.2	35	22.3
Living in the same apartment since birth	43	37.4	58	36.9
Allergic sensitization				
≥ 1 RAST +	115	100	0	
RAST *Der p1* +	48	41.7	0	
RAST cat +	30	26.1	0	
RAST birch +	39	33.9	0	
RAST gras +	84	73.0	0	
RAST *Cladosporium* +	14	12.2	0	
Allergic symptoms[a]				
Sneezing attacks	16	13.9	4	2.6
Red eyes	19	16.5	5	3.2
Runny or stuffed nose	16	13.9	8	5.2
Red eyes and runny or stuffed nose	5	4.4	0	0.0
Persistent wheezing	8	7.5	3	2.0
Itching rash	33	29.2	14	9.1
Allergic diseases				
Asthma[b,c]	17	14.8	0	0.0
Asthma attacks[a]	4	3.6	0	0.0
Hay fever[b]	12	10.4	1	0.7
Eczema[b]	23	20.0	14	9.0

[a]In past 12 months. [b]Lifetime diagnosis. [c]Doctor's diagnosis of asthma, asthmoid bronchitis, or spastic bronchitis.

Results

Study population. Table 1 presents characteristics of the study group, including the distribution of allergic sensitization (RAST-CAP ≥ 1), allergic symptoms, and diseases of sensitized cases and nonsensitized, nonasthmatic controls. We observed allergic sensitization [defined as testing positive for one specific IgE (> 0.35 kU/L) , i.e. for *Der p1,* cat, birch, grass, or *Cladosporium*] for 115 children referred to as cases. Our study sample consists of 150 boys and 122 girls because more boys tested positive for specific IgE. Parents of children who were sensitized and thus belonged to the case group self-reported being atopic slightly more often (25.2% vs. 17.2%) than parents of nonsensitized children, but we observed no difference in parental reports concerning dampness of homes and residential mobility since birth of the child in both groups.

Viable mold spore contamination of homes. The average weight of house dust taken from living room floors was 0.90 ± 0.85 g/m^2 and was similar for case and control homes (0.87 ± 1.01 for cases; 0.92 ± 0.71 for controls), yet we observed a large variation in mold levels between households. Table 2 presents the distribution of number of CFUs obtained per gram of dust taken in winter for total (all species) mold spores and selected groups of the most common molds found in homes. Although CFU values varied greatly, we found only one sample with > 10^6 CFU/g dust (76 × 10^6 CFU/g dust). Furthermore, no sample was free of molds. Restricting the period of analysis to winter samples only (November–April) meant that more than 60% of our samples were negative for *Alternaria* spores, and spore frequency was low in all samples positive for *Alternaria* (geometric mean 26; 95% CI, 16–43; 90th percentile, 10,000 CFU/g dust). Thus, we present results only for three genera of mold spores found most commonly in wintertime—*Cladosporium, Penicillium*, and *Aspergillus*. Figure 1 shows the cumulative frequency of the mold concentrations in the homes of cases and controls.

Cladosporium and *Penicillium* species were the most prevalent mold genera, but all three molds were positively correlated with and contributed to our total viable mold spore counts in the wintertime (r = 0.52 for *Cladosporium,* 0.49 for *Penicillium*, and 0.43 for *Aspergillus*) in both case and control homes (Table 3).

Furthermore, *Cladosporium* levels were not or were only weakly correlated with the indoor mold species *Aspergillus* and *Penicillium* in both seasons, suggesting that in the homes we studied two different patterns of mold growth contributed to overall high levels of mold spores; in the first type of home we found the typical indoor species *Aspergillus* and *Penicillium* commonly growing on foodstuffs and houseplants (*30*), and in the second type of home the dominant species was *Cladosporium*, an outdoor fungus that grows on textiles and foodstuffs when it gains access to the indoor environment.

Allergic sensitization and mold spore counts in household dust. We examined the association between allergic sensitization of children and mold spore counts in household dust in multiple logistic regression models adjusting the odds ratios for sensitization (at least one RAST-CAP positive test)

Table 2. Distribution of CFU per gram dust for total and selected genera of mold spores on living room floors of 272 households in eastern Germany.

Molds	Sensitized cases (n = 115)	Controls (n = 157)
Total molds		
Geometric mean	81,367	71,118
95% CI	70,562–93 827	61,285–82,530
n < LOD (%)	0 (0%)	0 (0%)
25th Percentile	50,000	45,000
Median	85,000	75,000
90th Percentile	210,000	195,000
Cladosporium		
Geometric mean	2,997	1,501
95% CI	1,531–5,867	804–2,804
n < LOD (%)	19 (16.5%)	35 (22.3%)
25th Percentile	5,000	5,000
Median	12,500	10,000
90th Percentile	40,000	30,000
Penicillium		
Geometric mean	2,041	2,014
95% CI	942–4,425	1,049–3,866
n < LOD (%)	26 (22.6%)	35 (22.3%)
25th Percentile	5,000	5,000
Median	15,000	15,000
90th Percentile	55,000	50,000
Aspergillus		
Geometric mean	856	245
95% CI	399–1,836)	117–513
n < LOD (%)	31 (27.0%)	65 (41.4%)
25th Percentile	LOD	LOD
Median	5,000	5,000
90th Percentile	30,000	25,000

LOD, Limit of detection.

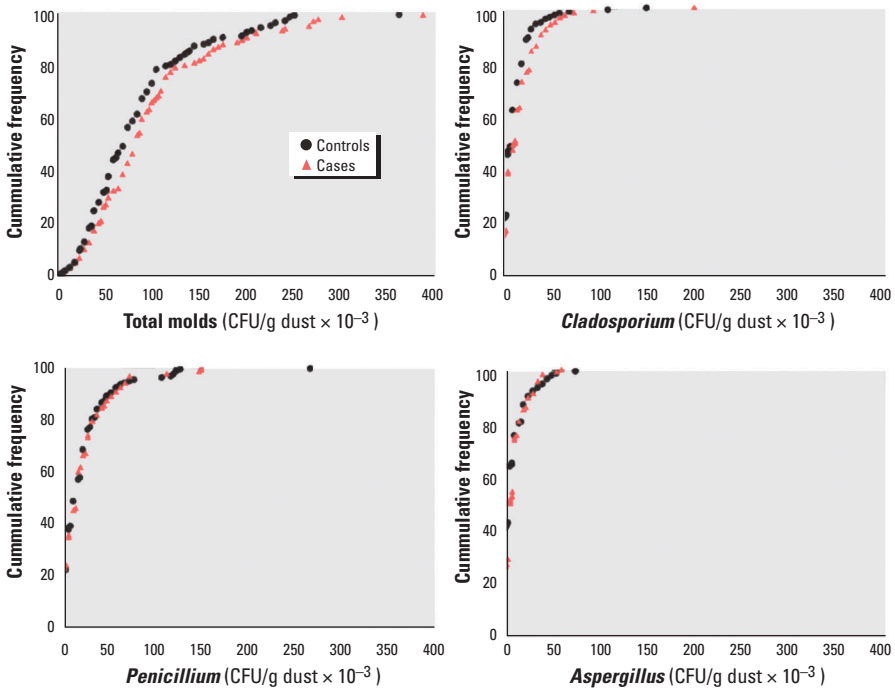

Figure 1. Cumulative frequency of the concentrations of molds in the homes of cases and controls.

by age, sex, residential region, parental education, and parental history of atopy.

High levels of *Cladosporium* (35,000 CFU/g dust or > 90th percentile) in wintertime household dust approximately tripled the risk of allergic sensitization in children (OR ≥ 90th percentile, 2.93; 95% CI, 1.17–7.36). *Aspergillus* spores increased the risk of allergic sensitization at a somewhat lower level [i.e., when the spore count increased above 25,000 CFU/g dust (25th percentile; OR 25th–90th percentile, 2.11; 95% CI, 1.22–3.65; OR ≥ 90th percentile: 1.76; 95% CI, 0.73–4.28] (Table 4). Sensitization of exposed children, however, was not limited to *Cladosporium* (specific IgE positive for *Cladosporium*). Rather, children exposed to increased viable mold levels were more likely to be sensitized to other allergens as well, such as pollen, cat, or house dust mites, similar to what has been reported previously (*17*). Considering mite allergen exposure as a potential confounder, we included also *Der p1* and *Der f1* levels into the model, but the results did not change (data not shown).

For *Penicillium* and also for total molds counts, we found slightly increased sensitization risks with exposure at high levels of mold spores in winter, but our effect estimates were imprecise and included the null value. In summer, however, *Penicillium* was the most important indoor contributor to overall sensitization (OR ≥ 90th percentile, 2.83; 95% CI, 1.25–6.44; data not shown).

Our results suggest a positive trend for risk of general allergic sensitization—not just to mold allergens—when children are exposed to mold spores. When restricting the analyses to children who lived in the same apartment since birth (n = 101), odds ratios increased for *Aspergillus* counts and showed a dose–response pattern (OR 25th–90th percentile, 2.21; 95% CI, 0.83–5.90; OR ≥ 90th percentile, 3.14; 95% CI, 0.63–15.7). Effects were also observed for high levels of *Cladosporium* (OR ≥ 90th percentile, 4.21; 95% CI, 0.72–24.7) and total molds counts (OR ≥ 90th percentile, 2.53; 95% CI, 0.52–12.4), but because of the loss in study subjects, the 95% confidence intervals included the null value. We did not observe consistent or strong associations with wintertime *Penicillium* spore counts in house dust (Figure 2).

Allergic symptoms and diseases and mold spore counts in household dust. Sensitized cases exposed to high levels of viable mold spores (> 90th percentile) were more likely to suffer from symptoms of rhinoconjunctivitis, including pink eye and runny and/or congested nose (OR 10.8 for total molds, OR 19. 8 for *Cladosporium*, and OR 23.8 for *Penicillium*; Table 5). We did not have enough subjects to draw a conclusion about the occurrence of other atopic and allergic symptoms and diseases, but in general high mold spore counts of any type seemed to increase symptom prevalence to some degree.

Discussion

Distribution of molds. Studies from Germany (*31*), Sweden (*9*), Denmark, the Netherlands (*22*), the United Kingdom (*32*), and Michigan (USA) (*24*) reported that *Penicillium* was the most prevalent indoor mold genus, followed by *Cladosporium*, whereas *Aspergillus* was the most commonly isolated indoor mold in Israel (*17*). Although the molds *Cladosporium* spp. and *Alternaria* spp. are generally considered outdoor species, they are also commonly found indoors. Outdoor mold levels vary greatly with season, and these variations may also contribute to variations in indoor levels of these molds. Therefore, we restricted our analyses of indoor dust samples to those taken in winter (November–April), when *Cladosporium* and *Alternaria* are less likely to grow outdoors. *Aspergillus* and *Penicillium* are the two most frequently encountered genera of indoor molds.The number of CFUs per gram of settled house dust is generally higher than the number measured in air samples because samples of settled dust probably reflect a cumulative measure of mold spores in homes. The number of CFUs per gram of dust found in our study was higher than those reported from other studies that used surface sampling methods (*24*). Our geometric mean of CFUs per gram dust for total molds was 81,367 in the group of sensitized cases and 71,118 in controls. Verhoeff et al. (*22*) sampled settled dust from

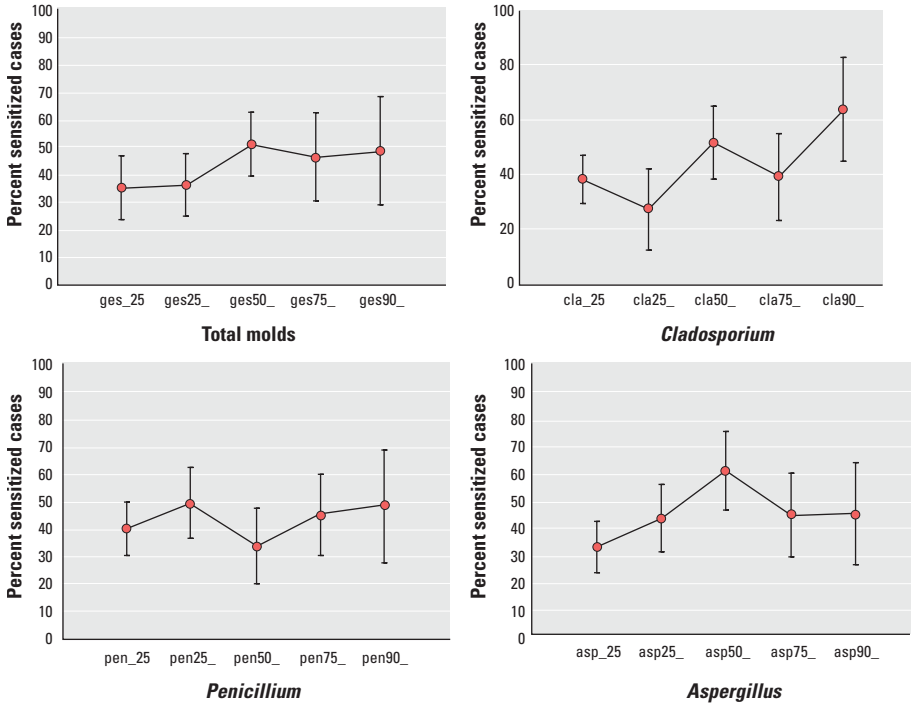

Figure 2. Percentage of sensitized children in the quartiles of exposure to molds (cases and controls).

Table 3. Spearman correlation coefficient matrix of the concentration of the mold spores on living room floors in winter of 272 households of children in eastern Germany.

	Controls			
Cases	Total molds	*Cladosporium*	*Penicillium*	*Aspergillus*
Total Molds				
r	1.0	0.47	0.49	0.37
p-Value		0.0001	0.0001	0.0001
Cladosporium				
r	0.55	1.0	–0.003	0.11
p-Value	0.0001		0.975	0.254
Penicillium				
r	0.48	0.09	1.0	0.27
p-Value	0.0001	0.240		0.003
Aspergillus				
r	0.44	0.19	0.25	1.0
p-Value	0.0001	0.019	0.002	

mostly noncarpeted bedroom floors over 2 months (October–November) and reported 8,300 CFU/g dust in homes of children with respiratory symptoms and 9,940 CFU/g dust in control household samples. Wickmann (*9*) reported a mean of only 1,000 CFU/g dust sampled from living room floors in late winter (February–March). Total numbers of CFUs per gram dust from carpets are significantly higher than for smooth floors (*22*), and 97% of our samples were taken from carpeted floors, which may explain the differences.

However, comparisons of quantitative and qualitative results from different studies are of limited value because studies not only used different sampling techniques for the same mold spores, but each study also focused on the identification of unique and different sets of mold spores (*33,34*).

Molds and allergic sensitization. Sensitization to molds is a risk factor for allergic diseases (*8,9*), and molds can be important indoor allergens (*17*). Reports of prevalence of allergic sensitization to molds vary widely ranging from 2% to 30% in subjects with respiratory allergy (*35*). The great variability in reported prevalence could derive from differences in environmental conditions, such as the geoclimatic areas under investigation, differences in population sensitivity, and differences in the characteristics and properties of diagnostic tests used to assess allergen extracts (*33*). The number of mold allergens for which reliable tests are available is small compared to other allergen extracts such as mites. Furthermore, isolation, purification, and standardization of allergens produced by molds are a major problem contributing to measurement error of unknown size in all studies.

The likelihood of developing sensitivity to aeroallergens depends on the degree of atopic susceptibility, the concentration and potency of allergens one is exposed to, and adjuvant factors (*36*). As did Garrett et al. (*37*), we found that winter exposure to high concentrations of mold spores such as *Cladosporium* increased allergic sensitization. Garrett et al. (*37*) also reported that atopy was significantly associated with *Aspergillus*. In our study, effects were most consistently observed for the species of *Cladosporium* and *Aspergillus*, where exposure above the 90th percentile increased the risk of allergic sensitization approximately 2- or 3-fold (*Cladosporium*, OR, 2.93; 95% CI, 1.17–7.36; *Aspergillus*, OR, 2.11; 95% CI, 1.22–3.65). Exposure to high levels of *Penicillium* (> 55,000 CFU/g dust, > 90th percentile) elevated the risk for allergic sensitization in winter only slightly, but *Penicillium* was the dominant indoor mold allergen in summer.

Although we conducted analyses stratifying for season (summer and winter), these analyses were not always informative because the sample was small. Summer total mold counts were dominated by high counts for the outdoor molds *Cladosporium* and *Alternaria*, and counts for these species correlated only weakly with the counts for the indoor molds *Pencillium* and *Aspergillus* (data not shown). We also observed that indoor *Cladosporium* measures were much higher in summer than in winter (median of 35,000 CFU/g in summer vs. 10,000 CFU/g in winter), while an opposite but weaker seasonal pattern was found for *Aspergillus* and *Penicillium,* supporting our notion that at least two different patterns of mold contamination of homes exist in the geographic area we studied. The latter two molds were more abundant in our winter samples.

Our winter results did not change when we adjusted for summertime spore counts from the same households or when we adjusted for house dust mite allergens (results not shown), and our results were strengthened when we restricted the analyses to children living in the same home since birth, but sample size and statistical efficiency was limited for this and other types of subgroup analyses (e.g., multiple logistic regression models examining sensistization to specific instead of all allergens; data not shown). As in our study, Garrett et al. (*37*) reported an elevated risk of general sensitization to allergens such as dust mites and dog allergens when they found high levels of viable *Cladosporium* and *Penicillium* spores in the air of homes in wintertime. Also similar to our results, these associations weakened when Garrett and co-workers instead used spore samples collected in late spring (*37*). This may be related to the known seasonal variability of mold spores in outdoor air (i.e., in winter levels of viable mold spore contamination in homes depend mostly on indoor factors because it is unlikely that spores are carried in from outdoors).

Molds and allergic symptoms. High indoor mold exposure (> 90th percentile) seems to contribute to allergic symptoms and diseases in both sensitized and nonsensitized children; however, because numbers in the nonsensitized group were small, effect estimates were imprecise or even nonestimable in this subgroup. This might suggest that both inflammatory allergic mechanisms, including type III allergy to mold-specific antigens and nonimmune inflammatory reactions to mold components, might be important (*18*). It is not clear which inflammatory and/or allergic mechanisms primarily

Table 4. Associations between exposure to fungal spores and prevalence of allergic sensitization.

Molds	(CFU/g dust)	Sensitized cases	Controls	Crude OR (95% CI)	Adjusted OR[a] (95% CI)
Total molds					
≤ 25th Percentile	≤ 48.750	24	44	1.0	1.0
> 25th/≤ 90th Percentile	48.750–200.000	79	100	1.45 (0.81–2.58)	1.56 (0.85–2.86)
> 90th Percentile	> 200.000	12	13	1.69 (0.67–4.29)	1.67 (0.65–4.29)
Cladosporium					
≤ 25th Percentile	≤ 5.000	45	72	1.0	1.0
> 25th/≤ 90th Percentile	5.000–35.000	54	76	1.14 (0.68–1.89)	1.15 (0.67–1.95)
> 90th Percentile	> 35.000	16	9	2.84 (1.16–6.98)	2.93 (1.17–7.36)
Penicillium					
≤ 25th Percentile	≤ 5.000	40	60	1.0	1.0
> 25th/≤ 90th Percentile	5.000–55.000	64	85	1.13 (0.68–1.89)	1.09 (0.64–1.84)
> 90th Percentile	> 55.000	11	12	1.38 (0.55–3.42)	1.38 (0.54–3.51)
Aspergillus					
≤ 25th Percentile	LOD	31	65	1.0	1.0
> 25th/≤ 90th Percentile	LOD–25.000	72	77	2.06 (1.16–3.35)	2.11 (1.22–3.65)
> 90th Percentile	> 25.000	12	15	1.68 (0.70–4.01)	1.76 (0.73–4.28)

LOD, limit of detection.
[a]Adjusted for age, sex, residential region, parental education, and parental atopy.

Table 5. Adjusted odds ratios for atopic symptoms and diseases and indoor mold spore counts.

	OR for fungal exposure above the 90th percentile[a]			
Atopic symptoms	Total molds	*Cladosporium*	*Penicillium*	*Aspergillus*
Sneezing attacks[b]	3.47 (1.07–11.3)	1.43 (0.36–5.58)	2.12 (0.52–8.63)	NE
Red eyes and runny or congested nose[b]	11.3 (1.23–103.1)	15.5 (2.08–1154.0)	17.6 (1.69–183.4)	NE
Persistent wheezing[b]	0.82 (0.10–7.13)	1.18 (0.47–2.94)	2.55 (0.44–14.7)	2.15 (0.41–11.4)
Itching rash[b]	1.46 (0.54–3.96)	1.18 (0.41–3.39)	0.62 (0.17–2.24)	1.47 (0.55–3.93)
Asthma[c,d]	0.47 (0.06–3.90)	0.52 (0.06–4.27)	1.74 (0.35–8.73)	1.29 (0.27–6.18)
Hay fever[c]	2.14 (0.39–11.8)	1.89 (0.35 –10.3)	2.57 (0.54–12.3)	NE
Eczema[c]	1.65 (0.57–4.81)	0.54 (0.12–2.44)	1.21 (0.38–3.88)	2.16 (0.80–2.52)

NE, not estimable.
[a]Adjusted for age, sex, region of residency, parental education, and parental atopy. [b]In past 12 months. [c]Lifetime. [d]Asthma, asthmoid bronchitis, or spastic bronchitis.

account for the presumed pathogenic effects of mold exposure (*18*). Nevertheless, allergic sensitization to mold spores plays a major role in atopy (*14*).

Existing studies suggest that exposure to allergens during a sensitive period in early life may enhance the risk of sensitization in genetically predisposed children (*35*), implying that for children with a positive family history, lower allergen concentrations may be sufficient to achieve sensitization (*1*). We did not observe clear patterns for increased sensitization risk in children with a positive history of parental atopy (data not shown), but the number of children in this group was quite small (n = 56), and we sampled mold spores in homes only for children older than 5 years of age.

Sampling technique and identification methods. The presence of molds in indoor environments is generally assessed using air or surface samples (*13*). Air sampling of viable mold particles usually is restricted to short periods of several hours and does not provide reliable data concerning the contamination by and growth of molds in nonindustrial indoor environments (*34*), especially because airborne samples are strongly influenced by outdoor levels of molds. High sampling variation has been observed for total airborne spore burden in repeated samples taken in the same home, possibly caused by domestic activity, cleaning, and ventilation (*22*). Assessment of viable mold particles (mold propagules) in settled house dust might be a useful measure of longer-term and cumulative exposure to indoor molds and is less influenced by indoor activities and turbulence. We used a simple, settled-dust sampling technique of standardized vacuuming of floor dust in the living room to measure viable mold particles.

To date, analysis of housedust samples to identify viable mold particles has not been standardized. Recently, a comparative study of 10 different analytic methods, however, showed that direct plating of dust onto DG18 agar was one of the more sensitive methods (*27*). In fact, use of DG18 agar produced higher numbers of CFU for all mold spores.

As an alternative to sampling mold spores, participants in previous studies of allergic and asthmatic diseases have often been asked to report dampness and odors as a surrogate for indoor mold exposures (*14*). Awareness of the existence of such exposures, however, may have caused overreporting of symptoms in exposed subjects and thus may have led to response bias. Furthermore, air sampling and cultivation of spores from house dust samples show only a modest agreement with such self-reported exposures (*11,37*). In the present study, relying on dust samples avoided reporting biases. But confounding bias may have occurred due to the fact that we included sensitized asthmatics in our case goup, and homes of asthmatics may be cleaned more rigorously to avoid symptoms. When we excluded from our analyses 17 children who were both asthmatics and sensitized, our results did not change.

We standardized our method of house dust sampling. We also believe that settled dust may be the best proxy for long-term exposures to mold allergens in the home environment. Furthermore, approximately 30% of all children for whom we collected samples had lived in the same home since birth and may have been exposed to high levels of mold throughout their lives.To explore fully any exposure–response relation between allergic sensitization and exposure to indoor molds, it is necessary to recruit sufficient individuals with high and low exposure levels. Although we found a wide range of CFUs per gram of dust in the homes of our study subjects, our overall sample size was relatively small and further reduced when we restricted our analyses to dust samples taken during wintertime to minimize the effect of seasonal variability in molds.

Conclusions

Our results suggest that indoor mold spore exposure, mainly during winter, might increase the risk of sensitization to all allergens in children. These findings are limited by methodologic difficulties of quantifying molds and by the relatively small number of homes studied. For future research we encourage using a longitudinal study design with a larger number of cases to allow analyses for allergen-specific instead of total sensitization. However, we found that allergic sensitization was significantly associated with exposure to one or more genera of indoor mold spores, even after adjustment for house dust mite exposure. The effect strengthened when we restricted our study population to children who had lived in the same home since birth. Furthermore, our study suggests that high indoor spore counts might increase the prevalence of allergic symptoms in all children whether they are sensitized or not.

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