Infant origins of childhood asthma associated with specific molds

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Background: The specific cause or causes of asthma development must be identified to prevent this disease.
Objective: Our hypothesis was that specific mold exposures are associated with childhood asthma development.
Methods: Infants were identified from birth certificates. Dust samples were collected from 289 homes when the infants were 8 months of age. Samples were analyzed for concentrations of 36 molds that comprise the Environmental Relative Moldiness Index (ERMI) and endotoxin, house dust mite, cat, dog, and cockroach allergens. Children were evaluated at age 7 years for asthma based on reported symptoms and objective measures of lung function. Host, environmental exposure, and home characteristics evaluated included a history of parental asthma, race, sex, upper and lower respiratory tract symptoms, season of birth, family income, cigarette smoke exposure, air conditioning, use of a dehumidifier, presence of carpeting, age of home, and visible mold at age 1 year and child's positive skin prick test response to aeroallergens and molds at age 7 years.
Results: Asthma was diagnosed in 24% of the children at age 7 years. A statistically significant increase in asthma risk at age 7 years was associated with high ERMI values in the child's home in infancy (adjusted relative risk for a 10-unit increase in ERMI value, 1.8; 95% CI, 1.5-2.2). The summation of levels of 3 mold species, *Aspergillus ochraceus*, *Aspergillus unguis*, and *Penicillium variabile*, was significantly associated with asthma (adjusted relative risk, 2.2; 95% CI, 1.8-2.7).
Conclusion: In this birth cohort study exposure during infancy to 3 mold species common to water-damaged buildings was associated with childhood asthma at age 7 years. (J Allergy Clin Immunol 2012;■■■:■■■-■■■.)

Key words: *Asthma, molds, speciation, infants, Environmental Relative Moldiness Index*

Most studies of the effect of mold exposure on asthma use case-control or cross-sectional methodology, with few prospective studies of asthma development.[1-3] The present investigation is a substudy of the prospective Cincinnati Childhood Allergy and Air Pollution Study (CCAAPS), which addresses the role of aeroallergens and diesel exhaust particles in the development of atopic respiratory tract disorders.[4,5]

Exposure to mold has been linked to asthma exacerbations, and evidence is increasing that mold might be a trigger for the development of asthma.[6,7] However, relevant mold species, as well as concentrations, timing, and durations of the exposure that might be contributory, are unknown. In some studies mold genera, such as *Alternaria*,[8] *Aspergillus*,[9] *Penicillium*,[10-12] *Aureobasidium*,[13] and *Cladosporium*,[14] were associated with respiratory illness, allergy, and/or asthma. These genera contain hundreds of species and are unlikely to have equal effects on health outcomes. The lack of practical methods to identify and quantify specific mold species is an unmet need.

Epidemiologic studies have typically used one of 3 methods to estimate mold exposures: inspection, counting/culturing of molds, and/or immunoassays for certain antigens. Inspection, counting, and/or culturing methods are neither standardized nor practical to identify and quantify mold species for large epidemiologic studies.[15] Immunologic assays aiming at estimating mold exposures are based on measuring antibody levels for certain mold proteins or antigens in environmental samples. These antigens are often cross-reactive with other molds.[16] Until specific mold species are identifiable, the mold allergens that are most relevant to respiratory disease cannot be recognized among thousands of possibilities.[17,18]

A DNA-based technology, mold-specific quantitative PCR (MSQPCR),[19] can be used to identify and quantify molds that are common in homes.[15,20] On the basis of the analysis of 36 molds in standardized dust samples from a random national sampling of homes, the Environmental Relative Moldiness Index (ERMI) was developed by US Environmental Protection Agency (EPA) and Department of Housing and Urban Development researchers.[20] In the ERMI methodology the results of species that are common to water-damaged homes (group 1) and those that are common to all homes (group 2) are summed into a single number, as described elsewhere.[20] In the United States 99% of ERMI values are between −10 and 20; the highest quartile is

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Abbreviations used
aRR: Adjusted relative risk
ATS: American Thoracic Society
AUC: Area under the curve
CCAAPS: Cincinnati Childhood Allergy and Air Pollution Study
EPA: Environmental Protection Agency
ERMI: Environmental Relative Moldiness Index
GAM: Generalized additive modeling
ICC: Interclass correlation coefficient
MCCT: Methacholine challenge test
MSQPCR: Mold-specific quantitative PCR
QIC: Quasilikelihood under independence model criteria
RR: Relative risk
SPT: Skin prick test

greater than 5.[20] Previously, we reported that higher ERMI home values (>5.2) in the homes of 176 of the CCAAPS children during infancy was predictive of the development of asthma at age 7 years.[21] The present study uniquely focused on quantifying individual mold species and had 2 objectives: (1) to assess the relationship of early exposure to specific molds on the development of childhood asthma and (2) to address the optimum grouping of the ERMI molds for predicting asthma by applying novel statistical methods to the grouping of the 36 species.

METHODS

Study population

Full-term infants born in Cincinnati, Ohio, and northern Kentucky between 2001 and 2003 were recruited by using birth certificate data. Eligibility for the study required that at least 1 parent was atopic, which was defined as having allergic symptoms and a positive skin prick test (SPT) response to at least 1 of 15 aeroallergens (meadow fescue, timothy, white oak, maple, American elm, red cedar, short ragweed, *Alternaria* species, *Aspergillus fumigatus*, *Penicillium* species, *Cladosporium* species, cat, dog, German cockroach, and house dust mite).[5,22] The 289 subjects identified for this analysis had to meet 2 eligibility requirements, including having sufficient stored dust samples from the age 1 year residence to perform all of the speciation analyses, and the children had to have completed the clinical examination at age 7 years. The study was approved by the Institutional Review Board of the University of Cincinnati.

Clinical evaluations

During a clinical visit conducted at age 7 years, children underwent SPTs for milk, egg, and the aforementioned 15 aeroallergens.[5,22] Children with a positive reaction to at least 1 aeroallergen at age 7 years were classified as having positive SPT responses. A questionnaire on the infants' respiratory symptoms was administered to the parent or caregiver. At age 7 years, the diagnosis of asthma was made based on asthma symptoms and objective measures of lung function and airway hyperresponsiveness.

All children completed spirometric testing (Koko; nSpire Health, Longmont, Colo) according to American Thoracic Society (ATS) criteria.[23] Predicted values of FEV_1, forced vital capacity, and their ratio (FEV_1/forced vital capacity) were calculated by using the equations of Wang et al,[24] as recommended by the ATS for children less than 8 years of age. A subset of children was assessed for airway reversibility by administering 2.5 mg of inhaled levalbuterol (Xopenex; Sunovion, Marlborough, Mass) through a nebulizer followed after 15 minutes by repeat spirometry if 1 of the following criteria was met: (1) parental report of the child's asthma symptoms in the previous 12 months (tight or clogged chest or throat in the past 12 months, difficulty breathing or wheezy after exercise, wheezing or whistling in the chest in the previous 12 months, or a previous doctor's diagnosis of asthma); (2) predicted FEV_1 of less than 90%; or (3) exhaled nitric oxide level of 20 ppb or greater. Exhaled nitric oxide (NIOX Flex; Aerocrine, Solna, Sweden) levels were measured according to ATS recommendations.[23] Children with a less than 12% increase in FEV_1 after administration of levalbuterol were tested for bronchial hyperresponsiveness by using a methacholine challenge test (MCCT) at a follow-up clinic visit. A modified 4-dose ATS protocol[25] was used with sequential methacholine concentrations of 0.0625, 0.25, 1, and 4 mg/mL. A positive MCCT result was defined as a 20% or greater decrease in FEV_1 after saline diluent challenge in response to 4 mg/mL or less of methacholine. Children were defined as having asthma if the parent reported asthma symptoms (as previously defined) and the child demonstrated either significant airway reversibility (≥12% increase in FEV_1) or a positive MCCT result.

On-site home visit and exposure assessment

Age 1 year on-site home visits were performed by 2-person teams when the infants were, on average, 8 months old. Information was collected on home characteristics, and floor dust samples were obtained for exposure assessment of indoor aeroallergens and mold.[26] Homes were categorized into 3 groups (no/low/high mold) based on observations of visible mold, water damage, and moldy odor.[26] In addition, homes were categorized into 2 groups based on the presence of water damage (yes/no). Dust samples were collected by vacuuming, large dust particles were removed by sieving (355-μm sieve), and the fine dust was stored at −20°C before analyses.[26]

Analysis of endotoxin and allergens

Dust samples were analyzed for endotoxin by using the Limulus Amebocyte Lysate assay (Pyrochrome LAL; Associates of Cape Cod, East Falmouth, Mass), as described earlier.[27-29] Endotoxin concentrations were expressed as endotoxin units per milligram of dust. The lower detection limit for endotoxin was 0.002 endotoxin units/mg. The concentrations in all measured dust samples were greater than the lower detection limits.

House dust mite (Der f 1), cat (Fel d 1), and cockroach (Bla g 1) allergens were analyzed by using mAbs, and dog allergen was analyzed by using polyclonal antibodies.[30] Results were expressed as nanograms of allergen/antigen (or IU for cockroach) per milliliter of extract and converted to micrograms of allergen/antigen per gram of sieved dust (concentration). The minimum value of detectable concentration was determined from each run of allergen analysis and varied as follows: 5 to 78 ng/mL for house dust mite, 1 to 12.5 ng/mL for cat, 12 to 391 ng/mL for dog, and 0.02 to 0.16 IU/mL for cockroach.

DNA extraction from dust and MSQPCR analysis

Methods and assays have been reported previously for performing MSQPCR analyses.[31,32] All primer and probe sequences, as well as known species comprising the assay groups, are available at http://www.epa.gov/microbes/moldtech.htm. Quality control results on repeated dust sampling and ERMI analysis are presented in the Methods section in this article's Online Repository at www.jacionline.org.

Statistical analyses

Analyses for objective 1. Preliminary analyses were performed in which associations between asthma at age 7 years and each predictor variable at age 1 year, including infant and family factors, home characteristics, and exposure variables, were evaluated by using univariate log-binomial regression. Predictor variables significant at the 15% level were included in an initial multiple regression model. These were removed by using stepwise regression. The ERMI and endotoxin values were modeled as continuous predictors. Dust allergens were dichotomized as greater than or less than the limit of detection because of the high percentages of values less than the limit of detection, ranging from 40% to 83%. Each multiple regression model included predictor variables that were significant at the 5% level. Sex was also included because it has been identified as a predictor of asthma in other studies. Approximately 61% of families moved to a different home between

the age 1 year home assessment and the age 7 years clinic visit. Therefore the multiple regression models were reanalyzed, including household moving status (moved/not moved).

Analyses for objective 2. The predictive values of continuously measured individual mold species and groupings of mold species were assessed by using multiple methods. First, mean values of $\log_e$ transformed concentrations ($\log_e$ spores · mg^{-1} dust) of the 36 individual mold species were compared between homes of asthmatic and nonasthmatic children. The species with means that differed significantly between the asthma and nonasthma groups were identified. The Holm method was applied to control the false discovery rate of the analyses, with statistical significance set at a false discovery rate of 0.05.[33] The second method was a cluster analysis, which was conducted to regroup the 36 individual mold species into clusters determined by using a nonhierarchic clustering mechanism specified by the oblique principal component cluster analysis in the SAS procedure PROC VARCLUS (SAS Institute, Cary, NC). A third method used a forward stepwise logistic regression analysis to identify those species that were most predictive of asthma. In addition, a random forest model was used for comparison with the above models.

The ERMI values, the group 1 and group 2 species separately, and the individual species that were identified as significant in the modeling were subsequently analyzed by using log binomial regressions with asthma as the outcome. Before performing these analyses, the linearity versus nonlinearity of each species or species combination was investigated by using generalized additive modeling (GAM) of asthma, assuming a binomial distribution.

In the GAMs restricted cubic spline functions were constructed, and significance levels of linear and nonlinear components of the spline functions of species data were obtained. The predictive ability of each species model was determined from the calculation of the areas under the curve (AUCs), which were obtained from receiver operating characteristics curve analyses of sensitivities and specificities obtained by regressing asthma status on the predicted values of asthma obtained from the GAM analyses. The highest AUC value corresponded to the best species model. After GAM models were reviewed, the associations between asthma status and each continuously modeled species or species combination were analyzed by using log binomial regression. The minimization of the quasilikelihood under independence model criteria (QIC) was used to identify the most predictive species model. The combination of species that corresponded to the model that was identified as "best" by using both criteria was included in a multiple regression model that included predictor variables that were significant at the 5% level and also sex.

Generalized estimating equation methodology with robust estimation of SEs was used to analyze all regression models. SAS 9.2 and R software were used for the analyses. *P* values of .05 or less were considered to indicate statistical significance unless stated otherwise.

RESULTS

Of the 289 children aged 7 years meeting the study criteria, 24% (n = 69) were given a diagnosis of asthma (see Table E1 in this article's Online Repository at www.jacionline.org). The percentages of African American children were 33% in the asthma group and 20% in the nonasthma group, with a combined percentage of 22% (n = 66). For the asthma and nonasthma groups, the percentages of children with at least 1 parent reporting asthma were 61% and 42%, respectively, with a combined percentage of 46% (n = 134). There were no significant differences among the distributions of parental asthma, sex, race, and income between the 289 children in this substudy and the entire age 7 years cohort of 617 children (data not shown). However, the asthma rate was lower in the entire age 7 years cohort (16%, $P < .01$). A similar pattern was observed between the subgroup of 289 children and the rest of the 617-children cohort (n = 328); only asthma rate was different ($P < .001$). A statistically significant increase in the risk of asthma was associated with race (African American vs other), low family income (<$20,000 vs ≥$40,000 per year), parental asthma, upper respiratory tract symptoms at age 1 year, and positive SPT responses to any aeroallergen and to mold at age 7 years. Sex, season of birth, and the presence/absence of household cigarette smoke exposure (either at age 1 or 7 years), as well as home characteristics at age 1 year (use of central air conditioning, home dehumidifiers; presence of carpeting; visible mold; water damage; and age of home), were not significantly associated with asthma (see Table E1). The mean age 1 ERMI value of the homes of asthmatic patients was 4 times higher than that of the homes of nonasthmatic patients (6.7 vs 1.5), and increasing ERMI values were associated with increased risk of asthma (relative risk [RR], 1.6 [95% CI, 1.4-1.9] for a 10-unit increase in ERMI value). Cat allergen was significantly and inversely associated with asthma. Endotoxin, dust mite, dog, and cockroach allergens, however, were not significant (see Table E2 in this article's Online Repository at www.jacionline.org). ERMI values were not significantly associated with mold sensitization (RR, 0.9; 95% CI, 0.4-1.9).

The first multivariate model in Table I (model 1) shows that the risk of asthma at age 7 years was 1.8 times greater for a 10-unit increase in age 1 ERMI values (adjusted relative risk [aRR],

TABLE I. aRRs and 95% CIs obtained from multiple regression models relating predictor variables to asthma at age 7 years for 289 subjects

Predictor variable determined at age 1 y	aRR*	95% CI
Model 1		
ERMI	1.8	1.5-2.2
Parental asthma (yes vs no)	1.7	1.3-2.1
Income (<$40,000 vs ≥$40,000)		
<$20,000	1.4	1.1-1.7
$20,000~$40,000	1.4	1.00-1.9
>$40,000	Referent	
Cat allergen (≥LOD vs <LOD)	0.5	0.3-0.7
SPT response to any aeroallergen at age 7 y (positive vs negative)	1.5	1.2-2.0
Sex (male vs female)	1.1	0.9-1.4
Upper respiratory tract symptoms (yes vs no)†	2.2	1.6-3.1
Model 2		
Summation of 3 species‡	2.2	1.8-2.7
Parental asthma (yes vs no)	1.4	1.1-1.8
Income		
<$20,000	1.4	1.02-1.8
$20,000~$40,000	1.4	1.1-1.8
>$40,000	Referent	
Cat allergen (≥LOD vs <LOD)	0.6	0.4-0.9
SPT response to any aeroallergen at age 7 y (positive vs negative)	1.7	1.3-2.1
Sex (male vs female)	1.1	0.8-1.4
Upper respiratory tract condition (yes vs no)†	2.5	1.7-3.7

Predictors were measured at age 1 year, except SPT responses, which were measured at age 7 years. Among 289 children, 69 (24%) had asthma at age 7 years. Note: initial models included ERMI value, race, sex, parental asthma, income, cigarette smoking, central air-conditioning, endotoxin, cat allergen, and SPT responses to any aeroallergen.

LOD, Limit of detection.

*The aRR corresponds to an interquartile range (−2.7 to 7.1) increase in ERMI value, and the aRR for a 10-unit increase was the same as for an interquartile increase. End points of 95% CIs indicate the range of aRRs compatible with data about 95% of the time.

†"Upper respiratory tract symptoms" was coded yes when any of ear or sinus infection, strep throat, or tonsillitis was present.

‡Species include *A ochraceus*, *A unguis*, and *P variabile*.

TABLE II. Geometric means (coefficient of variation)* of mold species at age 1 year for 289 subjects

Molds	Asthmatic patients	Nonasthmatic patients
Group 1		
1. *Aspergillus flavus*	2.3 (6.1)	1.4 (3.7)
2. *Aspergillus fumigatus*	6.5 (3.1)	4.3 (2.9)
3. *Aspergillus niger*	**13.7 (6.8)**	**5.7 (4.5)**
4. *Aspergillus ochraceus*	**6.8 (3.8)**	**2.0 (3.2)**
5. *Aspergillus penicillioides*	25.6 (2.4)	19.5 (2.4)
6. *Aspergillus restrictus*	1.7 (3.8)	1.2 (4.2)
7. *Aspergillus sclerotiorum*	2.4 (4.0)	1.6 (7.5)
8. *Aspergillus sydowii*	2.0 (8.2)	0.9 (10.8)
9. *Aspergillus unguis*	**2.6 (7.2)**	**1.0 (6.2)**
10. *Aspergillus versicolor*	5.5 (7.8)	1.8 (7.2)
11. *Aureobasidium pullulans*	4599.4 (1.5)	3891.3 (6.1)
12. *Chaetomium globosum*	3.8 (4.8)	2.0 (4.3)
13. *Cladosporium sphaerospermum*	137.2 (2.1)	70.5 (3.4)
14. *Eurotium* group	124.7 (5.7)	59.7 (10.0)
15. *Paecilomyces variotii*	6.6 (6.7)	3.2 (2.4)
16. *Penicillium brevicompactum*	20.6 (2.7)	14.6 (4.7)
17. *Penicillium corylophilum*	1.0 (6.2)	0.7 (5.8)
18. *Penicillium crustosum*	1.6 (5.1)	1.2 (14.2)
19. *Penicillium purpurogenum*	0.8 (7.2)	0.6 (4.3)
20. *Penicillium spinulosum*	1.1 (2.9)	0.9 (7.4)
21. *Penicillium variabile*	**12.6 (4.0)**	**4.0 (9.0)**
22. *Scopulariopsis brevicaulis*	3.7 (6.7)	1.8 (4.1)
23. *Scopulariopsis chartarum*	2.0 (2.9)	1.3 (4.5)
24. *Stachybotrys chartarum*	3.3 (2.0)	2.1 (3.4)
25. *Trichoderma viride*	14.3 (2.9)	9.3 (4.4)
26. *Wallemia sebi*	85.2 (7.2)	43.2 (3.7)
Group 2		
27. *Acremonium strictum*	1.9 (1.8)	1.8 (3.5)
28. *Alternaria alternata*	262.3 (1.8)	216.6 (12.7)
29. *Aspergillus ustus*	5.2 (3.7)	2.5 (3.6)
30. *Cladosporium cladosporioides* 1	2099.3 (1.5)	1349.2 (1.8)
31. *Cladosporium cladosporioides* 2	28.1 (3.1)	27.7 (1.9)
32. *Cladosporium herbarum*	232.0 (1.9)	186.9 (1.7)
33. *Epicoccum nigrum*	315.9 (1.7)	245.2 (3.7)
34. *Mucor* group	97.5 (3.2)	61.3 (3.9)
35. *Penicillium chrysogenum* 2	51.1 (1.7)	31.2 (8.4)
36. *Rhizopus stolonifer*	2.0 (4.5)	1.5 (3.5)

Results in boldface are significantly different between asthmatic and nonasthmatic patients at the 5% level after adjustment using the Holm method.
*The coefficient of variation (ie, SD/mean) was calculated on the original scale.

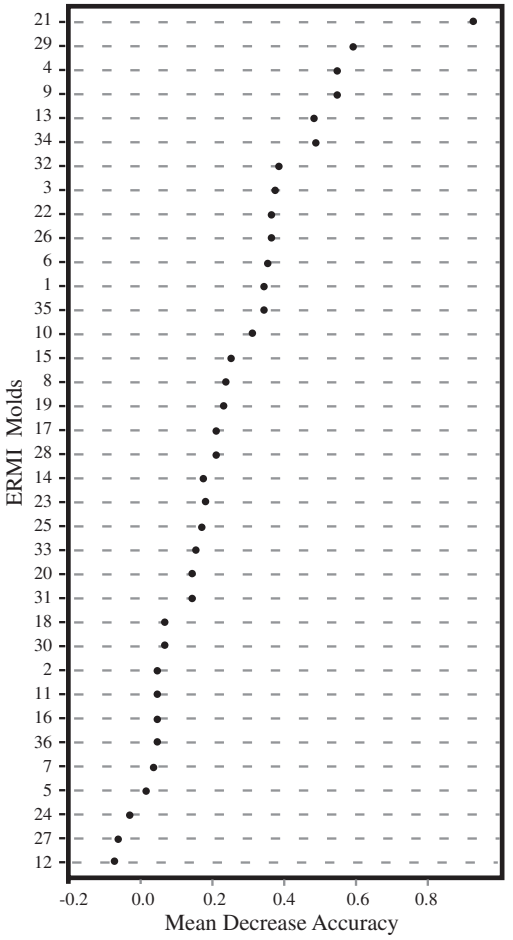

FIG 1. Change in root mean square error for the 36 mold species from random forest analysis. The numbers on the *y-axis* refer to species as presented in Table II (eg, no. 21 is *P variabile*, no. 29 is *A ustus*, no. 4 is *A ochraceus*, and no. 9 is *A unguis*). The higher the mean decrease in accuracy, the more important the species is for the classification between asthmatic and nonasthmatic patients.

1.8; 95% CI, 1.5-2.2). Among the other covariates, parental asthma (aRR, 1.7; 95% CI, 1.3-2.1), low income (<$20,000; aRR, 1.4; 95% CI, 1.1-1.7), positive SPT response to any aeroallergen at age 7 years (aRR, 1.5; 95% CI, 1.2-2.0), and upper respiratory tract infections at age 1 year (aRR, 2.2; 95% CI, 1.6-3.1) were strong risk factors for asthma. In contrast, cat allergen measured in settled house dust at age 1 year reduced the risk of asthma development (aRR, 0.5; 95% CI, 0.3-0.7). The results were essentially the same when the model included the moving status of the family (not shown).

Table II shows that among the ERMI species, levels of the group 1 mold species *Aspergillus niger*, *A ochraceus*, *A unguis*, *and P variabile* were significantly different between asthma groups ($P < .05$, after adjustment for multiple comparisons by using the Holm method). The results of the stepwise regression showed that the following 4 species were the most predictive of asthma ($P < .05$, results not shown): *A ochraceus*, *A unguis*, *P variabile*, and *Scopulariosis brevicaulis*. Random forest analysis showed that the most predictive species were *A ochraceus* (no. 4), *A unguis* (no. 9) *P variabile* (no, 21), and *Aspergillus ustus* (no. 29, Fig 1). In summary, the 3 methods were in agreement in identifying *A ochraceus*, *A unguis*, and *P variabile* as the most predictive of asthma development.

The cluster analysis identified 6 clusters (see Table E3 in this article's Online Repository at www.jacionline.org), one of which predominantly consisted of group 2 species (6/8 species, cluster B), and the other that was predominately group 1 species (which were combined as cluster A, see Table E4 in this article's Online Repository at www.jacionline.org). Cluster A was significantly and positively associated with asthma, whereas cluster B was not associated with asthma (Table III, model 3).

In separate analyses, even alone, the group 1 molds were still predictive of asthma. However, the group 2 species were not significantly associated with asthma development (Table III). Table III also provides the QIC and AUC values for each of the models. A smaller QIC indicates a better model fit, whereas a higher AUC indicates a better ability for predicting asthma. The AUC was highest (73%) and the QIC was lowest (292) for model 4, which was obtained by summing the 3 species identified to be

TABLE III. Comparisons of different models for predicting asthma based on (1) ERMI value, (2) groups of ERMI species (group 1, group 2, and both), (3) nonhierarchic clustering (cluster A, 28 molds; cluster B, 8 molds), (4) 3 asthma-associated species (each separately or summed), and (5) ERMI without 3 asthma-associated species*

	RR (95% CI)	QIC†	AUC (%)‡
1. ERMI	1.6 (1.4-1.9)	300	69
2. ERMI groups			
2A. Group 1 species alone summed	1.8 (1.6-1.9)	295	70
2B. Group 2 species alone summed	1.3 (0.95-1.7)	315	58
2C. Groups 1 and 2 separately in the same model			
Group 1 species summed	1.9 (1.6-2.2)	296	71
Group 2 species summed	0.8 (0.6-1.2)		
3. Cluster analysis			
3A. Cluster A (28 species summed)§	1.8 (1.4-2.4)	295	68
3B. Cluster B (8 species summed)§	1.3 (0.97-1.7)	312	60
4. Asthma-associated species‖			
All 3 species summed	1.8 (1.3-2.4)	292	73
5. ERMI without those 3 species	1.4 (1.2-1.8)	306	61

*For each of the models 1, 2A, 2C, 3A, 4, and 5, the linear component was significant ($P < .01$) and the nonlinear linear component was not significant ($P > .10$).
†QIC is a criterion of model fit, in which smaller values indicate a better fit.
‡Higher values indicate better predictive value for the model.
§*Aspergillus sydowii*, *Aureobasidium pullulans*, *Aspergillus strictum*, *Alternaria alternata*, *Cladosporium cladosporioides* type 1, *C cladosporioides* type 2, *Cladosporium herbarum*, and *Epicoccum nigrum* belong to cluster B; other 28 species belong to cluster A.
‖*Aspergillus ochraceus*, *Aspergillus unguis*, and *Penicillium variabile*.

most predictive for asthma (*A ochraceus*, *A unguis*, and *P variabile*). The AUC for ERMI (Table III, model 1) was 69%. The ERMI value was reanalyzed to assess how much the sum of the 3 species accounted for the prediction of asthma (model 5). Without the 3 species, the AUC was decreased to 61%.

Because the best fit and highest predictive value was found for the model that included the summation of the 3 mold species, the combined effect of these 3 mold species was investigated in a multivariate model. The 3 species remained significantly associated with asthma in the final multivariate model (aRR, 2.2; 95% CI, 1.8-2.7, Table I, model 2).

DISCUSSION

This study is unique because we have identified specific molds associated with asthma development in a prospective study, arriving at a consensus from different models. A DNA-based method of mold analysis and multiple modeling and statistical approaches demonstrated that 3 mold species, *A ochraceus*, *A unguis*, and *P variabile*, were significantly associated with asthma development. The ERMI metric itself, the group 1 molds, and the combination of the 3 named species were significantly associated with asthma. However, asthma was best predicted with the 3 species alone. Group 2 species were not associated with asthma development.

Cluster analysis created groupings very similar to the established ERMI groups 1 and 2. These findings support the current approach for calculating the mold burden in homes as described by the ERMI metric. The group 2 molds are distributed throughout the United States[34] and originate primarily from the outside environment. By subtracting the sum of the logs of the concentrations of the 10 group 2 molds, a baseline is created with which to compare homes. This approach minimizes mold population differences that result from family cleaning habits, the degree to which open windows are used for ventilation, or other processes that could affect the inside-to-outside environmental interchange.

Although molds are ubiquitous in our environment, not all are found commonly indoors. The 3 molds identified as relevant to childhood asthma are typically found in water-damaged homes as opposed to homes without water damage.[35-37] Furthermore, mold exposure, as described by higher ERMI values in infants' homes, was predictive of a child having asthma at age 7 years after adjusting for other risk factors. For a 10-unit increase in the ERMI scale, the risk of asthma increased 80%. These results strengthen the findings in our previous study, in which a 2-fold increased risk for high versus low ERMI values was seen in a smaller subgroup of CCAAPS children.[21]

In this cohort only 12% of asthmatic patients were sensitized to molds (*Alternaria* species, *Aspergillus fumigatus*, *Penicillium* species, or *Cladosporium* species), although 58% were sensitized to an aeroallergen at age 7 years. Furthermore, ERMI values at age 1 year were not associated with mold sensitization at age 7 years. There are 2 possible explanations for the low rate of mold sensitization. First, none of the molds we have identified as potentially relevant are standard parts of commercially available mold skin testing panels. Second, as an alternative explanation, it is possible, although unproved, that mold exposure might contribute to asthma through nonallergic mechanisms.[38]

The results described here do not prove that these specific molds cause asthma but are still intriguing and provide impetus to correct residential water problems in the homes of especially high-risk infants. Water damage and mold growth can potentially be found in any home. We have previously demonstrated that mold culture data are not adequate to describe the mold burden in homes.[39] In the current study visible mold was not associated with asthma, which might be due to hidden mold problems. In another analysis of exposures of this age 7 years cohort, we did not find consistent associations between visible mold damage categories and microbial measurements.[40] In the American Healthy Homes survey the homeowner was not aware and an inspector did not detect mold in homes with ERMI values of greater than 5 in about 50% of homes.[41] Furthermore, remediation of homes with high ERMI values has been shown to improve children's asthma.[42] Therefore ERMI analysis appears to be a more sensitive method than visual inspection to detect health-relevant mold exposure.

Future prospective studies might benefit from examining the limitations of this study, one of which is the small sample size. However, because prospective studies take many years (9 years in this case), it is a significant challenge and expense to maintain a large cohort over time. Another limitation is that the 36 mold species identified and quantified likely represented only a fraction of the total molds in these samples. Therefore using more of the MSQPCR assays will likely promote the discovery of additional relevant molds. The 3 mold species identified thus far should provide a starting point for targeted laboratory studies. Although the study included a comprehensive list of home and family characteristics and environmental exposures, there could be other confounding variables, such as the mother's vitamin D level or stress level during pregnancy.

In conclusion, infant exposures to specific mold species were statistically correlated with asthma development at age 7 years

after controlling for other potential risk factors. Expecting parents with a family history of asthma might find it prudent to correct any water and mold problems in the home. Mold assessment using the ERMI might help in identifying the high-risk homes.

Clinical implications: Remediation of infants' homes for water damage and mold might mitigate some cases of asthma. Therapeutics for asthma could be more efficient if targeted toward specific mold species.

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METHODS

Repeatability of ERMI measurement

The reproducibility of the ERMI measurement, when repeated for the same home, was assessed by calculating the interclass correlation coefficient (ICC) between initial ERMI values and a quality control sample of repeated values. Quality control repeat sampling was conducted in 5% of randomly selected homes of the age 7 years cohort within 3 months of the initial dust sampling. Measures of intrahome and interhome variability were obtained by analyzing a random intercept model. The ICC value was calculated as the ratio of intrahome to total (interhome + intrahome) variability. The analysis was implemented with SAS PROC MIXED, version 9.2. The ICC value was 0.61, which indicates moderate-to-high repeatability.[E1]

Previous studies have shown lower ICC values for repeatability of indoor microbial measurements. Hyvärinen et al[E2] reported an ICC value of 0.51 for floor dust endotoxin and 0.36 for airborne endotoxin. Crawford et al[E3] found an ICC of 0.10 for airborne fungal spores and an ICC of 0.09 for airborne (1-3)-β-D-glucan.

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TABLE E1. Unadjusted asthma RRs and 95% CIs for predictor variables

Predictor variables determined at age 1 y	Asthma group (n = 69)	Nonasthma group (n = 220)	RR* (95% CI)†
Demographics			
Sex, male	41 (59%)	127 (58%)	1.1 (0.7-1.6)
Race (African American/other), African American	23 (33%)	43 (20%)	1.7 (1.1-2.6)
Income			
<$20,000	20 (30%)	31 (14%)	2.1 (1.3-3.4)
$20,000~$40,000	14 (21%)	38 (18%)	1.5 (0.8-2.5)
>$40,000	33 (49%)	145 (68%)	Referent
Season of birth			
Spring	13 (19%)	37 (17%)	1.2 (0.6-2.1)
Summer	18 (26%)	44 (20%)	1.3 (0.8-2.2)
Fall	16 (23%)	63 (29%)	0.9 (0.5-1.6)
Winter	22 (32%)	76 (34%)	Referent
Parental asthma, yes	42 (61%)	92 (42%)	1.8 (1.2-2.8)
Upper respiratory tract symptoms, yes‡	50 (77%)	122 (58%)	2.0 (1.2-3.4)
Lower respiratory tract symptoms, yes§	36 (55%)	118 (56%)	0.98 (0.6-1.5)
Home characteristics			
Air conditioning, yes	47 (68%)	174 (79%)	0.7 (0.4-1.01)
Dehumidifier, yes	14 (20%)	35 (16%)	1.2 (0.7-2.1)
Carpet, yes	51 (74%)	175 (80%)	0.8 (0.5-1.3)
Age of home (year built)			
<1955	33 (48%)	83 (38%)	1.6 (0.9-3.0)
1955-1985	24 (35%)	80 (36%)	1.3 (0.7-2.5)
>1985	12 (17%)	57 (26%)	Referent
Home visible mold			
0 = none	32 (47%)	103 (47%)	1.4 (0.5-3.5)
1 = low	32 (47%)	98 (45%)	1.4 (0.6-3.6)
2 = high	24 (6%)	19 (9%)	Referent
Water damage, yes	15 (22%)	23 (20%)	1.1 (0.7-1.8)
Household cigarette smoke,‖ yes	23 (33%)	50 (23%)	1.5 (0.97-2.3)
Allergic sensitization¶			
SPT response positivity, yes	39 (58%)	87 (40%)	1.7 (1.1-2.6)
Mold positivity, yes	8 (12%)	10 (5%)	2.0 (1.1-3.5)

Numbers in each group and percentages of column totals are shown.

*RRs correspond to an increase for the specified category compared with the reference category.

†End points of 95% CIs indicate the range of RRs compatible with data about 95% of the time.

‡"Upper respiratory tract symptoms" was coded yes when any of ear or sinus infection, strep throat, or tonsillitis was present.

§"Lower respiratory tract symptoms" was coded yes when any of flu, cough, croup, cysts, bronchitis/ bronchiolitis, viral infection, or pneumonia was present.

‖RR of 1.5 (95% CI, 0.97-2.3) for household cigarette smoke at age 7 years.

¶Allergic sensitization was determined at age 7 years for 67 asthmatic and 215 nonasthmatic patients.

TABLE E2. Unadjusted asthma RRs and 95% CIs for exposure variables

Exposure variables at age 1 y	Asthma group (n = 69)	Nonasthma group (n = 220)	RR* (95% CI)†
ERMI, mean (SD)	6.7 (8.4)	1.5 (6.9)	1.6 (1.4-1.9)
Endotoxin (EU/mg), geometric mean (GSD)	85.6 (3.4)	66.2 (3.3)	1.2 (0.9-1.6)
Dust mite allergen (μg/g), geometric mean (GSD)	0.14 (12.1)	0.12 (10.6)	1.4 (0.9-2.3)
> LOD‡	21.7%	15.0%	
Cat allergen (μg/g), geometric mean (GSD)	0.39 (10.9)	0.93 (15.5)	0.5 (0.3-0.9)
>LOD‡	13.0%	26.9%	
Dog allergen (μg/g), geometric mean (GSD)	14.5 (7.9)	14.5 (9.1)	1.2 (0.8-1.9)
>LOD‡	65.2%	59.4%	
Cockroach allergen (IU/g)	0.9 (4.8)	1.01 (4.8)	0.8 (0.5-1.2)
>LOD‡	40.6%	46.9%	

Means and SDs of ERMI values and geometric means and geometric SDs *(GSD)* of endotoxin and dust allergens per unit of dust are shown. Note: numbers of subjects vary slightly because of missing data.

EU, Endotoxin unit; *LOD*, limit of detection.

*RR corresponds to an interquartile range increase for ERMI and $\log_e$ endotoxin values. The RR for a 10-unit increase was the same as that for an interquartile increase in ERMI values.

†End points of 95% CIs indicate the range of RRs compatible with data about 95% of the time.

‡Categories of dust allergens greater than and less than the limit of detection. Geometric means and SDs of dust allergens were calculated after values less than limits of detection were divided by 2.

TABLE E3. Cluster analysis: list of mold species in each cluster

Cluster	Mold species
Cluster 1	*Aspergillus fumigatus*
	Aspergillus ochraceus
	Aspergillus versicolor
	Paecilomyces variotii
	Penicillium corylophilum
	Penicillium crustosum
	Trichoderma viride
	Mucor group
	Penicillium chrysogenum 2
Cluster 2	*Aspergillus restrictus*
	Aspergillus unguis
	Chaetomium globosum
	Penicillium spinulosum
	Stachybotrys chartarum
	Aspergillus ustus
Cluster 3	*Aspergillus penicillioides*
	Cladosporium sphaerospermum
	Eurotium group
	Scopulariopsis brevicaulis
	Wallemia sebi
	Rhizopus stolonifer
Cluster 4	*Aspergillus flavus*
	Aspergillus niger
	Aspergillus sclerotiorum
	Penicillium brevicompactum
	Penicillium purpurogenum
	Scopulariopsis chartarum
Cluster 5	*Penicillium variabile*
Cluster 6	*Aspergillus sydowii*
	Aureobasidium pullulans
	Acremonium strictum
	Alternaria alternata
	Cladosporium cladosporioides 1
	Cladosporium cladosporioides 2
	Cladosporium herbarum
	Epicoccum nigrum

TABLE E4. Cluster analysis: mean values of mold concentrations in 6 clusters by asthma status

	Mean values of mold concentrations for each cluster by asthma status (cell equivalents/mg)		*P* value*
	Asthma group	Nonasthma group	
Clusters using VARCLUS			
Cluster 1†	56.7	10.9	<.001
Cluster 2†	6.4	0.05	<.001
Cluster 3†	16.6	13.3	<.01
Cluster 4†	6.4	5.2	<.01
Cluster 5†	2.2	1.8	<.001
Cluster 6‡	35.1	32.9	.08

**P* values were testing whether mean values were significantly different between the asthma and nonasthma groups.
†Because clusters 1 to 5 were significantly different between the asthma and nonasthma groups, these clusters were combined as cluster A for further analysis.
‡Cluster 6 was renamed cluster B for further analysis.