Published in final edited form as:

J Allergy Clin Immunol. 2012 September ; 130(3): 639–644.e5. doi:10.1016/j.jaci.2012.05.030.

Infant Origins of Childhood Asthma Associated with Specific Molds

Tiina Reponen, PhD^a, James Lockey, MS, MD^{a,b}, David I. Bernstein, MD^b, Stephen J. Vesper, PhD^c, Linda Levin, PhD^a, Gurjit K. Khurana Hershey, MD, PhD^d, Shu Zheng, PhD^a, Patrick Ryan, PhD^{a,d}, Sergey A. Grinshpun, PhD^a, Manuel Villareal, MD^b, and Grace LeMasters, PhD^a

^aUniversity of Cincinnati, Department of Environmental Health, Cincinnati, OH, USA

^bDepartment of Internal Medicine, University of Cincinnati, Cincinnati, OH, USA

^cEnvironmental Protection Agency, Cincinnati, OH, USA

^dCincinnati Children's Hospital Medical Center, Cincinnati, OH, USA

Abstract

Background—The specific cause(s) of asthma development must be identified in order 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 age eight months. 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 seven for asthma based on reported symptoms and objective measures of lung function. Host, environmental exposures and home characteristics evaluated included history of parental asthma, race, gender, upper and lower respiratory symptoms, season of birth, family income, cigarette smoke exposure, air conditioning, dehumidifier, carpeting, age of home, and visible mold at age one and child positive skin prick test (SPT) to aeroallergens and molds at age seven.

Results—Asthma was diagnosed in 24% of the children at age seven. A statistically significant increase in asthma risk at age seven was associated with high ERMI levels in the child's home in infancy (adjusted risk ratio (aRR) for a 10-unit increase in ERMI = 1.8, 95% CI=1.5, 2.2). The summation of levels of three mold species, *Aspergillus ochraceus*, *Aspergillus unguis*, and *Penicillium variable* was significantly associated with asthma (aRR = 2.2, 95% CI=1.8, 2.7).

Conclusion—In this birth cohort study, exposure during infancy to three mold species common to water-damaged buildings was associated with childhood asthma at age seven.

© 2012 American Academy of Allergy, Asthma and Immunology. Published by Mosby, Inc. All rights reserved.

Address reprint requests to Dr. Tiina Reponen, University of Cincinnati, Department of Environmental Health, P.O. Box 670056, Cincinnati, OH, 45267-0056, USA; Tel. 513-558-0571; Fax 513-5582263; Tiina.Reponen@uc.edu.

There are no financial interests to disclose.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

Keywords

Asthma; molds; speciation; infants; Environmental Relative Moldiness Index

INTRODUCTION

Most studies of the effect of mold exposure on asthma are case-control or cross-sectional, with few prospective studies of asthma development.^{1, 2, 3} The present investigation is a substudy of the prospective Cincinnati Childhood Allergy and Air Pollution Study (CCAAPS) that addresses the role of aeroallergens and diesel exhaust particles in the development of atopic respiratory disorders.^{4, 5}

Exposure to mold has been linked to asthma exacerbations, and evidence is increasing that mold may be a trigger for development of asthma.^{6, 7} However, relevant mold species as well as concentrations, timing and durations of the exposure that may be contributory are unknown. In some studies, mold genera such as *Alternaria*,⁸ *Aspergillus*,⁹ *Penicillium*,^{10, 11, 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.

Epidemiological studies have typically used one of three methods to estimate mold exposures: inspection, counting/culturing 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 epidemiological studies.¹⁵ Immunological assays aiming at estimating mold exposures are based on measuring antibodies 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} Based on 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 the United States Environmental Protection Agency (US EPA) and the Department of Housing and Urban Development (HUD) researchers.²⁰ In the ERMI methodology, the results of species that are common to water-damaged homes (Group 1) and those that are common in all homes (Group 2) are summed into a single number as described elsewhere.²⁰ In the USA, 99% of ERMI values are between -10 and 20; the highest quartile is above 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 seven.²¹ The present study uniquely focused on quantifying individual mold species and had two objectives. The first objective was to assess the relationship of early exposure to specific molds on development of childhood asthma. The second objective was 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 using birth certificate data. Eligibility for the study required that at least one parent was atopic, defined as having allergic symptoms and a positive skin prick test (SPT) to at least one of 15 aeroallergens (meadow fescue, timothy, white oak, maple, American elm, red cedar, short ragweed, *Alternaria spp.*, *Aspergillus fumigatus*, *Penicillium spp.*, *Cladosporium spp.*, cat, dog, German cockroach, and house dust mite).^{5, 23} The 289 subjects identified for this analysis had to meet two eligibility requirements including having sufficient stored dust samples from the age one residence to perform all of the speciation analyses, and the children had to have completed the clinical examination at age seven. The study was approved by the Institutional Review Board at the University of Cincinnati.

Clinical evaluations

During a clinical visit conducted at age seven, children underwent skin prick testing (SPT) for milk, egg and the aforementioned 15 aeroallergens.^{5,23} Children who showed a positive reaction to at least one aeroallergen at age seven were classified as SPT positive. A questionnaire on the infants' respiratory symptoms was administered to the parent or caregiver. At age seven, the diagnosis of asthma was made based on asthma symptoms and objective measures of lung function and airway hyperresponsiveness.

All children completed spirometry (Koko; nSpire Health, Inc.) testing according to American Thoracic Society (ATS) criteria.²⁴ Predicted values of the forced expiratory volume in 1 second (FEV1), forced vital capacity (FVC), and their ratio (FEV1/FVC) were calculated using the equations of Wang et al. as recommended by the ATS for children less than age eight.²⁵ A subset of children were assessed for airway reversibility by administering 2.5 mg of inhaled Xopenex® by nebulizer followed after 15 minutes by repeat spirometry if one 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-diagnosis of asthma), 2) predicted FEV1 < 90%, 3) exhaled nitric oxide > 20 ppb. Exhaled nitric oxide (NIOX Flex, Aerocrine Inc.) was measured following ATS recommendations.²⁴ Children with <12% increase in FEV1 following the administration of Xopenex were tested for bronchial hyperresponsiveness by methacholine challenge test (MCCT) at a follow-up clinic visit. A modified four-dose ATS protocol²⁶ was utilized with sequential methacholine concentrations of 0.0625, 0.25, 1, and 4 mg/mL. A positive MCCT was defined as a 20% or greater decline from FEV1 after saline diluent challenge in response to > 4 mg/ML 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 FEV1) or a positive MCCT.

On-site home visit and exposure assessment

Age one on-site home visits were performed by two-person teams when the infants were on average eight months old. Information was collected on home characteristics and floor dust samples were obtained for exposure assessment of indoor aeroallergens and mold.²⁷ Homes were categorized into three groups (no/low/high mold) based on observations of visible mold, water damage and moldy odor.²⁷ In addition, homes were categorized into two 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.²⁷

Analysis of endotoxin and allergens

Dust samples were analyzed for endotoxin using the *Limulus* Amebocyte Lysate assay (Pyrochrome LAL; Associates of Cape Cod Inc.), as described earlier.^{28,29, 30} Endotoxin concentrations were expressed as endotoxin units per mg of dust (EU/mg). The lower detection limit (LDL) for endotoxin was 0.002 EU/mg. The concentrations in all measured dust samples were above the LDLs.

House dust mite (Der f 1), cat (Fel d 1), and cockroach (Bla g 1) allergens were analyzed using monoclonal antibodies, and dog allergen was analyzed using polyclonal antibodies.³³ Results were expressed as ng allergens/antigens (or IU for cockroach) per mL extract, and converted to µg allergens/antigens per g of sieved dust (concentration, µg/g). The minimum value of detectable concentration was determined from each run of allergen analysis and varied as follows: 5–78 ng/mL for house dust mite, 1–12.5 ng/mL for cat, 12–391 ng/mL for dog, and 0.02–0.16 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 from <http://www.epa.gov/microbes/moldtech.htm>. Quality control results on repeated dust sampling and ERMI analysis are presented in the online supplement.

Statistical analyses

Analyses for objective one—Preliminary analyses were performed in which associations between asthma at age seven and each predictor variable at age one, including infant and family factors, home characteristics, and exposure variables, were evaluated by univariate log-binomial regression. Predictor variables, significant at the 15% level, were included in an initial multiple regression model. These were removed by stepwise regression. The ERMI and endotoxin values were modeled as continuous predictors. Dust allergens were dichotomized as above or below the limit of detection (LOD), due to the high percents of values below the LOD, ranging from 40% to 83%. Each multiple regression model included predictor variables which were significant at the 5% level. Gender was also included because it has been identified as a predictor of asthma in other studies. About 61% of families moved to a different home between the age one home assessment and age seven clinic visit. Therefore, the multiple regression models were reanalyzed including household moving status (moved/not moved).

Analyses for objective two—The predictive values of continuously measured individual mold species and groupings of mold species were assessed by 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 asthmatics' and non-asthmatics' homes. The species whose means differed significantly between asthma groups were identified. The Holm method was applied to control the false discovery rate (FDR) of the analyses, with statistical significance set at FDR=0.05.³⁴ The second method was a cluster analysis, which was conducted to regroup the 36 individual mold species into clusters, determined by a nonhierarchical clustering mechanism specified by the oblique principal component cluster analysis in the SAS procedure PROC VARCLUS. A third method utilized a forward stepwise logistic regression analysis to identify those species that were most predictive of asthma. In addition, a Random Forest model was utilized 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 log binomial regressions with asthma as the outcome. Prior to performing these analyses, the

linearity, versus nonlinearity, of each species or species combination was investigated by 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 (ROC) 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 combinations were analyzed by log binomial regression. The minimization of the QIC (Quasi-likelihood Under the Independence Model Criterion) was used to identify the most predictive species model. The combination of species that corresponded to the model that was identified as 'best' by both criteria was included in a multiple regression model that included predictor variables which were significant at the 5% level and also gender.

Generalized Estimating Equation (GEE) methodology with robust estimation of standard errors was used to analyze all regression models. SAS 9.2 and R software were utilized for the analyses. P-values ≤ 0.05 were considered to indicate statistical significance unless stated otherwise.

RESULTS

Of the 289 age seven children, meeting study criteria, 24% (n=69) were diagnosed with asthma (Table EI in the online repository). The percents of African American (AA) children were 33% in the asthma and 20% in the non-asthma groups with a combined percent of 22% (n= 66). For asthma and non-asthma groups, the percents of children with at least one parent reporting asthma were 61% and 42%, respectively, with a combined percent of 46% (n= 134). There were no significant differences between the distributions of parental asthma, gender, race and income between the 289 children in this substudy and the entire age seven cohort of 617 children (data not shown). However, the asthma rate was lower in the entire age seven cohort (16%) ($p<0.01$). Similar pattern was observed between the subgroup of 289 and the rest of the 617 cohort (n=328): only asthma rate was different ($p<0.001$). A statistically significant increase in the risk of asthma was associated with race (AA versus other, low family income ($< \$20$ K versus $\geq \$40$ K per year), parental asthma, upper respiratory symptoms at age one, as well as positive skin prick test (SPT) to any aeroallergen and SPT to mold at age seven. Gender, season of birth, and the presence/absence of household cigarette smoke exposure (either at age one or seven), as well as home characteristics at age one (use of central air conditioning and/or home dehumidifiers, presence of carpeting, visible mold, water damage and age of home) were not significantly associated with asthma (Table EI in the online repository). The mean age one ERMI value of the homes of asthmatics was four times higher than the homes of non-asthmatics (6.7 vs. 1.5) and increasing ERMI was associated with increased risk of asthma [Relative Risk =RR=1.6, 95% Confidence Interval (CI)=1.4,1.9 for a 10-unit increase in ERMI]. Cat allergen was significantly and inversely associated with asthma. Endotoxin, dust mite, dog and cockroach allergens, however, were not significant (Table EII in the online repository). ERMI was 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 seven was 1.8 times greater for a 10-unit increase in age one ERMI (adjusted RR=aRR=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 $< \$20$ K (aRR=1.4, 95% CI=1.1, 1.7), SPT positivity to any aeroallergen at age seven

(aRR=1.5, 95% CI=1.2–2.0), and upper respiratory infections at age one (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 one 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 moving status of the family (not shown).

Table II shows that among the ERMI species, Group 1 mold species, *Aspergillus niger*, *A. ochraceus*, *A. unguis*, and *P. variable* were significantly different between asthma groups ($p < 0.05$, after adjustment for multiple comparisons by the Holm method). The results of the stepwise regression showed that the following four species were the most predictive of asthma ($p < 0.05$, results not shown): *A. ochraceus*, *A. unguis*, *P. variable*, and *Scopulariopsis brevicaulis*. Random Forest analysis showed that the most predictive species were *A. ochraceus* (#4), *A. unguis* (#9), *P. variable* (#21), and *A. ustus* (#29) (Figure 1). In summary, the three methods were in agreement in identifying *A. ochraceus*, *A. unguis*, and *P. variable* as most predictive of asthma development.

The cluster analysis identified six clusters (Table EIII in the online repository), one of which predominantly consisted of Group 2 species (6/8 species) (Cluster B), and the others were predominately Group 1 species (which were combined as Cluster A) (Table EIV in the online repository). 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 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 three species identified to be most predictive for asthma (*A. ochraceus*, *A. unguis* and *P. variable*). The AUC for ERMI (Table III, Model 1) was 69%. The ERMI was reanalyzed to assess how much the sum of the three species accounted for the prediction of asthma (Model 5). Without the three species, the AUC was lowered to 61%.

As the best fit and highest predictive value was found for the model that included the summation of the three mold species, the combined effect of these three mold species was investigated in a multivariate model. The three 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 three mold species, *A. ochraceus*, *A. unguis*, and *P. variable*, were significantly associated with asthma development. The ERMI metric itself, the Group 1 molds and the combination of the three named species were significantly associated with asthma. However, asthma was best predicted with the three 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 US³⁸ and originate primarily from the outside environment. By subtracting the sum of the logs of the

concentrations of the ten Group 2 molds, a baseline is created with which to compare homes. This approach minimizes mold population differences that result from family cleaning habits, degree to which open windows are used for ventilation, or other process which could affect the inside to outside environmental interchange.

Although molds are ubiquitous in our environment, not all are found commonly indoors. The three molds identified as relevant to childhood asthma are typically found in water-damaged homes, as opposed to homes without water damage.^{35, 36, 37} Furthermore, mold exposure, as described by higher ERMI values in infants' homes, was predictive of a child developing asthma at age seven 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 finding in our previous study, in which two-fold increased risk for high versus low ERMI was found in a smaller subgroup of CCAAPS children.²¹

In this cohort, only 12% of asthmatics were sensitized to molds (*Alternaria spp.*, *Aspergillus fumigatus*, *Penicillium spp.*, or *Cladosporium spp.*) although 58% were sensitized to an aeroallergen at age seven. Furthermore, ERMI at age one was not associated with mold sensitization at age seven. There are two 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 unproven, that mold exposure may contribute to asthma through non allergic mechanisms.²²

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 may be due to hidden mold problems. In another analysis of exposures of this age seven cohort, we did not find consistent associations between visible mold damage categories and microbial measurements.⁴⁰ In the American Healthy Homes survey, neither the homeowner was aware of nor an inspector detected mold in homes with ERMI values above 5 in about 50% of homes.⁴¹ Furthermore, remediation of high ERMI homes 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 may benefit from examining the limitations of this study, one of which is the small sample size. However, because prospective studies take many years (nine 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 three mold species identified so far should provide a starting point for targeted laboratory studies. Although the study included comprehensive list of home and family characteristics and environmental exposures, there could be other confounding variables, such as mother's vitamin D-level or stress level during pregnancy.

CONCLUSION

Infant exposures to specific mold species were statistically correlated with asthma development at age seven after controlling for other potential risk factors. Expecting parents with a family history of asthma may find it prudent to correct any water and mold problems in the home. Mold assessment using ERMI may help in identifying the high risk homes.

Acknowledgments

This study was partially supported by the U. S. Department of Housing and Urban Development Grant No. OHLHH0226-10 and the National Institute of Environmental Health Sciences (NIEHS) Grant No. RO1 ES11170 awarded to the University of Cincinnati.

The U.S. Environmental Protection Agency (EPA) through its Office of Research and Development, partially funded and collaborated in the research described here. It has been subjected to the Agency's peer review and has been approved as an EPA publication. Mention of trade names or commercial products does not constitute endorsement or recommendation by the EPA for use. Commercial use of the ERMI technology can provide royalties to the EPA.

Abbreviations

AA	African American
aRR	adjusted Relative Risk
ATS	American Thoracic Society
AUC	Area Under the Curve
CCAAPS	Cincinnati Childhood Allergy and Air Pollution Study
CI	Confidence Interval
ELISA	Enzyme-linked Immunosorbent Assay
ERMI	Environmental Relative Moldiness Index
FDR	False discovery rate
FEV1	Forced expiratory volume in one second
FVC	Forced vital capacity
GEE	Generalized Estimating Equation
HUD	Department of Housing and Urban Development
MCCT	Methacholine challenge test
MSQPCR	Mold specific quantitative polymerase chain reaction
QIC	Quasi-likelihood Under Independence Model Criteria
ROC	Receiver Operating Characteristic
SPT	Skin Prick Test
US EPA	United States Environmental Protection Agency

References

1. Wickman M, Ahlstedt S, Lilja G, van Hage Hamsten M. Quantification of IgE antibodies simplifies the classification of allergic diseases in 4-year-old children. A report from the prospective birth cohort study--BAMSE. *Pediatr Allergy Immunol.* 2003; 14:441–7. [PubMed: 14675470]
2. Belanger K, Beckett W, Triche E, Bracken MB, Holford T, Ren P, et al. Symptoms of wheeze and persistent cough in the first year of life: associations with indoor allergens, air contaminants, and maternal history of asthma. *Am J Epidemiol.* 2003; 158:195–202. [PubMed: 12882940]
3. Jaakkola JJ, Hwang BF, Jaakkola N. Home dampness and molds, parental atopy, and asthma in childhood: a six-year population-based cohort study. *Environ Health Perspect.* 2005; 113:357–61. [PubMed: 15743728]

4. Ryan PH, LeMasters G, Biagini J, Bernstein D, Grinspun SA, Shukla R, et al. Is it traffic type, volume, or distance? Wheezing in infants living near truck and bus traffic. *J Allergy Clin Immunol.* 2005; 16:279–84. [PubMed: 16083780]
5. Ryan PH, Lemasters GK, Biswas P, Levin L, Hu S, Lindsey M, et al. A comparison of proximity and land use regression traffic exposure models and wheezing in infants. *Environ Health Perspect.* 2007; 115:278–84. [PubMed: 17384778]
6. Institute of Medicine, National Academies of Science. *Damp Indoor Spaces and Health.* The National Academies Press; 2004. p. 355
7. WHO Europe (World Health Organization Europe). *WHO guidelines for indoor air quality: Dampness and mould.* World health Organization; Copenhagen, Denmark: 2009.
8. Salo PM, Arbes SJ Jr, Sever M, Jaramillo R, Cohn RD, London SJ, et al. Exposure to *Alternaria alternata* in US homes is associated with asthma symptoms. *J Allergy Clin Immunol.* 2006; 118:892–8. [PubMed: 17030243]
9. Agarwal R, Gupta D. Severe asthma and fungi: current evidence. *Med Mycol.* 2011; 49(Suppl 1):S150–7. [PubMed: 20662637]
10. Gent JF, Ren P, Belanger K, Triche E, Bracken MB, Holford TR, et al. Levels of household mold associated with respiratory symptoms in the first year of life in a cohort at risk for asthma. *Environ Health Perspect.* 2002; 110:A781–6. [PubMed: 12460818]
11. Bundy KW, Gent JF, Beckett W, Bracken MB, Belanger K, Triche E, et al. Household airborne *Penicillium* associated with peak expiratory flow variability in asthmatic children. *Ann Allergy Asthma Immunol.* 2009; 103:26–30. [PubMed: 19663123]
12. Rosenbaum PF, Crawford JA, Anagnost SE, Wang CJ, Hunt A, Anbar RD, et al. 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.* 2010; 20:503–15. [PubMed: 19536075]
13. Stark PC, Celedón JC, Chew GL, Ryan LM, Burge HA, Muilenberg ML, et al. Fungal levels in the home and allergic rhinitis by 5 years of age. *Environ Health Perspect.* 2005; 113:1405–9. [PubMed: 16203255]
14. Garrett MH, Rayment PR, Hooper MA, Abramson MJ, Hooper BM. Indoor airborne fungal spores, house dampness and associations with environmental factors and respiratory health in children. *Clin Exp Allergy.* 1998; 28:459–67. [PubMed: 9641573]
15. Vesper S. Traditional mould analysis compared to a DNA-based method of mould analysis. *Crit Rev Microbiol.* 2011; 37:15–24. [PubMed: 20874612]
16. Schmechel D, Green BJ, Blachere FM, Janotka E, Beezhold DH. Analytical bias of cross-reactive polyclonal antibodies for environmental immunoassays of *Alternaria alternata*. *J Allergy Clin Immunol.* 2008; 121:763–8. [PubMed: 18036643]
17. Salo PM, Sever ML, Zeldin DC. Indoor allergens in school and day care environments. *J Allergy Clin Immunol.* 2009; 124:185–92. [PubMed: 19577284]
18. Heinrich J. Influence of indoor factors in dwellings on the development of childhood asthma. *Int J Hyg Environ Health.* 2011; 214:1–25. [PubMed: 20851050]
19. Haugland, R.; Vesper, S. Identification and quantification of specific fungi and bacteria. Patent 6,387,652 Granted. May 14. 2002
20. Vesper SJ, McKinstry C, Haugland RA, Wymer L, Ashley P, Cox D, et al. Development of an environmental relative moldiness index for homes in the U. S *J Occup Environ Med.* 2007; 49:829–33.
21. Reponen T, Vesper S, Levin L, Johansson E, Burkle J, Ryan P, et al. High Environmental Relative Moldiness Index during infancy as predictor of age seven asthma. *Ann Allergy Asthma Immunol.* 2011; 107:120–6. [PubMed: 21802019]
22. Mendell MJ, Mirer AG, Cheung K, Tong M, Douwes J. Respiratory and allergic health effects of dampness, mold, and dampness-related agents: a review of the epidemiologic evidence. *Environ Health Perspect.* 2011; 119:748–56. Online suppl. p. 118. [PubMed: 21269928]
23. LeMasters G, Wilson K, Levin L, Biagini J, Ryan PH, Lockey JE, et al. High prevalence of aeroallergen sensitization among infants of atopic parents. *J Pediatr.* 2006; 149:505–11. [PubMed: 17011322]

24. Miller MR, Hankinson J, Brusasco V, Burgos F, Casaburi R, Coates A, et al. ATS/ERS Task Force. Eur Respir J. 2005; 26:319–38. [PubMed: 16055882]
25. Wang X, Dockery DW, Wypij D, Gold DR, Speizer FE, Ware JH, et al. Am Rev Respir Dis. 1993; 148:1502–8. [PubMed: 8256891]
26. Crapo RO, Casaburi R, Coates AL, Enright PL, Hankinson JL, Irvin CG, et al. Guidelines for methacholine and exercise challenge testing-1999. This official statement of the American Thoracic Society was adopted by the ATS Board of Directors, July 1999. Am J Respir Crit Care Med. 2000; 161:309–29. [PubMed: 10619836]
27. Cho SH, Reponen T, Bernstein DI, Olds R, Levin L, Liu X, et al. The effect of home characteristics on dust antigen concentrations and loads in homes. Sci Total Environ. 2006; 371:31–43. [PubMed: 17049968]
28. Reponen T, Singh U, Schaffer C, Vesper S, Johansson E, Adhikari A, et al. Visually observed mold and moldy odor versus quantitatively measured microbial exposure in homes. Sci Tot Environ. 2010; 408:5565–74.
29. Campo P, Kalra HK, Levin L, Reponen T, Olds R, Lummus ZL, et al. Influence of dog ownership and high endotoxin on wheezing and atopy during infancy. J Allergy Clin Immunol. 2006; 118:1271–78. [PubMed: 17157656]
30. Iossifova Y, Reponen T, Bernstein D, Levin L, Zeigler H, Kalra H, et al. House dust (1–3)-beta-D-glucan and wheezing in infants. Allergy. 2007; 62:504–513. [PubMed: 17441791]
31. Haugland RA, Brinkman NE, Vesper SJ. Evaluation of rapid DNA extraction methods for the quantitative detection of fungal cells using real time PCR analysis. J Microbiol Meth. 2002; 50:319–23.
32. Haugland RA, Varma M, Wymer LJ, Vesper SJ. Quantitative PCR of selected *Aspergillus*, *Penicillium* and *Paecilomyces* species. Sys Appl Microbiol. 2004; 27:198–210.
33. Epstein TG, Bernstein DI, Levin L, Khurana Hershey GK, Ryan PH, Reponen T, et al. Opposing effects of cat and dog ownership and allergic sensitization on eczema in an atopic birth cohort. J Pediatr. 2011; 158:265–71. [PubMed: 20884006]
34. Holm S. A simple sequentially rejective multiple test procedure. Scand J Stat. 1979; 6:65–70.
35. Vesper SJ, McKinstry C, Yang C, Haugland RA, Kerckmar CM, Yike I, et al. Specific molds associated with asthma. J Occu Environ Med. 2006; 48:852–858.
36. Vesper S, McKinstry C, Haugland R, Neas L, Hudgens E, Heidenfelder B, et al. Higher Environmental Relative Moldiness Index (ERMIsm) values measured in Detroit homes of severely asthmatic children. Sci Total Environ. 2008; 394:192–196. [PubMed: 18280542]
37. Vesper SJ, McKinstry C, Ashley P, Haugland RA, Yeatts K, Bradham K, et al. Quantitative PCR Analysis of molds in the dust from homes of asthmatic children in North Carolina. J Environ Monit. 2007; 9:826–830. [PubMed: 17671663]
38. Vesper S, Wakefield J, Ashley P, Cox D, Dewalt G, Friedman W. Geographic distribution of Environmental Relative Moldiness Index (ERMI) molds in U.S. homes. J Environ Public Health. 2011:Article ID 242457. 11 pages. 10.1155/2011/242457
39. Meklin T, Haugland RA, Reponen T, Varma M, Lummus Z, Bernstein D, Wymer LJ, Vesper SJ. Quantitative PCR analysis of house dust can reveal abnormal mold conditions. J Environ Monit. 2004; 6:615–620. [PubMed: 15237292]
40. Reponen T, Singh U, Schaffer C, Vesper S, Johansson E, Adhikari A, Grinshpun SA, Indugula R, Ryan P, Levin L, LeMasters G. Visually observed mold and moldy odor versus quantitatively measured microbial exposure in homes. Sci Total Environ. 2010; 408:5565–74. [PubMed: 20810150]
41. Vesper S, McKinstry C, Cox D, Dewalt G. Correlation between ERMI values and other moisture and mold assessments of homes in the American Healthy Homes Survey. J Urban Health. 2009; 86:850–60. [PubMed: 19536652]
42. Kerckmar CM, Dearborn DG, Schluchter M, Xue L, Kirchner HL, Sobolewski J, Greenberg SJ, Vesper SJ, Allan T. Reduction in asthma morbidity in children as a result of home remediation aimed at moisture sources. Environ Health Perspect. 2006; 114:1574–80. [PubMed: 17035145]

Clinical Implications

Remediation of infants' homes for water damage and mold may mitigate some cases of asthma. Therapeutics for asthma may be more efficient if targeted towards specific mold species.

Figure 1. Change in root mean square error for the 36 mold species from Random Forest analysis. (The numbers on y-axis refer to species as presented in Table II, e.g. #21 is *Penicillium variable*, #29 is *Aspergillus ustus*, #4 is *Aspergillus ochraceus*, and #9 is *Aspergillus unguis*). The higher the mean decrease accuracy, the more important the species is for the classification between asthmatics and non-asthmatics.

Table I

Adjusted risk ratios (aRR) and ninety-five percent confidence intervals (95% CI) obtained from multiple regression models relating predictor variables to asthma at age seven for 289 subjects. Predictors were measured at age one, except SPT measured at age seven. Among 289 children, 69 (24%) had developed asthma at age seven.

Predictor Variable Determined at Age One	aRR ^a	(95% CI)
Model 1		
ERMI	1.8	(1.5, 2.2)
Parental asthma (yes vs no)	1.7	(1.3, 2.1)
Income (<\$40K vs >40K)		
<\$20K	1.4	(1.1, 1.7)
\$20K~\$40K	1.4	(1.00, 1.9)
>\$40K	Referent	
Cat allergen (LOD vs < LOD)	0.5	(0.3, 0.7)
SPT to any aeroallergen at age seven (positive vs. negative)	1.5	(1.2, 2.0)
Gender (male vs. female)	1.1	(0.9, 1.4)
Upper Respiratory Symptoms (yes vs. no) ^b	2.2	(1.6, 3.1)
Model 2		
Summation of 3 species ^c	2.2	(1.8, 2.7)
Parental asthma (yes vs. no)	1.4	(1.1, 1.8)
Income		
<\$20K	1.4	(1.02, 1.8)
\$20K~\$40K	1.4	(1.1, 1.8)
>\$40K	Referent	
Cat allergen (LOD vs < LOD)	0.6	(0.4, 0.9)
SPT to any aeroallergen at age seven (positive vs. negative)	1.7	(1.3, 2.1)
Gender (male vs. female)	1.1	(0.8, 1.4)
Upper Respiratory Condition (yes vs. no) ^b	2.5	(1.7, 3.7)

Note: Initial models included ERMI, race, gender, parental asthma, income, cigarette smoking, central air-conditioning, endotoxin, cat allergen, and SPT to any aeroallergen.

^a Adjusted RR corresponds to an interquartile range (−2.7, 7.1) increase for ERMI, the aRR for a 10-unit increase was the same as for interquartile increase. End points of 95% CI indicate the range of Risk Ratios compatible with data about 95% of the time.

^b "Upper Respiratory Symptoms" was coded yes when any of ear or sinus infection, strep throat or tonsillitis is yes.

^c Species include *Aspergillus ochraceus*, *Aspergillus unguis* and *Penicillium variable*.

Table II

Geometric means (coefficient of variation)^a of mold species at age one for 289 subjects. Bolded results are significantly different between asthma and non-asthma at the 5% level, after adjustment by the Holm method.

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

^a Coefficient of variation(=standard deviation/mean) was calculated on the original scale.

Table III

Comparisons of different models for predicting asthma based on (1) ERMI, (2) Groups of ERMI species (Group 1, Group 2 and both), (3) non-hierarchical clustering (Cluster A=28 molds and Cluster B=8 molds), (4) three asthma associated species (each separately or summed), and (5) ERMI without three asthma associated species.^a

	RR (95% CI)	QIC^b	AUC (%)^c
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) ^d	1.8 (1.4, 2.4)	295	68
3B. Cluster B (8 species summed) ^d	1.3 (0.97, 1.7)	312	60
4. Asthma associated species^e			
All three species summed	1.8 (1.3, 2.4)	292	73
5. ERMI without those three species	1.4 (1.2, 1.8)	306	61

^aFor each of the following models: 1, 2A, 2C, 3A, 4 and 5, the linear component was significant ($p < 0.01$), and the nonlinear linear component was not significant ($p > 0.10$).

^bQIC=Quasilikelihood under independence. QIC is a criterion of model fit, where smaller value indicates a better fit.

^cAUC=Area under the curve; higher value indicates better predictive value for the model.

^d*Aspergillus sydowii*, *Aureobasidium pullulans*, *Aspergillus strictum*, *Alternaria alternata*, *Cladosporium cladosporioides* type 1, *C. cladosporioides* type 2, *Cladosporium herbarum*, *Epicoccum nigrum* belong to Cluster B, other 28 species belong to Cluster A.

^e*Aspergillus ochraceus*, *Aspergillus unguis*, *Penicillium variabile*