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Abstract

Background Fungal exposures are believed to play an important role in the development of asthma and atopy, accounting for increased asthmatic symptoms and severe asthma exacerbation. Indoor fungal species vary both in taxa and concentration in different residences and in different regions.

Objectives We explored the fungal species spectrum in eighty-eight homes with at least one asthmatic child in the Middle West region of US mostly during late spring and fall season in comparison to eighty-five homes that did not contain an asthmatic child during flu season.

Methods The average fungal spore counts per cubic meter of air in the bedroom of the enrolled child, the main living spaces and outdoor environments, and the culturable fungal colony forming units per cubic meter of air samples in the main living space from each home were measured.

Results The results indicated that *Cladosporium*, *Penicillium*, *Aspergillus*, Basidiospores, *Epicoccum*, and *Pithomyces* were found in more asthmatic homes than in homes without an asthmatic child or existed in higher concentration in asthmatic homes than in homes without an asthmatic child even after adjusting outdoor spore concentration. The results for culturable fungal species confirmed most of these findings even after adjusting for seasonal factors. Although *Alternaria* was commonly found in both kinds of homes, there was no significant difference in detection rate or concentration of *Alternaria* between asthmatic homes and homes without an asthmatic child by either spore counting or culturable airborne detection.

Conclusion and Clinical Relevance Since many allergens have been identified in these fungal species, identifying and controlling these fungal species in asthmatic homes might be expected to improve asthma care and benefit asthmatic children.

Key words: Asthma, fungal exposure, home environment

INTRODUCTION

Asthma is a chronic inflammatory airway disease characterized by obstruction, which is associated with increased bronchial responsiveness to a variety of stimuli [1]. Environmental factors are believed to play an important role accounting for increased prevalence, incidence and frequency of asthma exacerbation in both children and adults especially for allergic asthma. The indoor environment may be the most important place for asthma care because asthmatic patients spend most of their daily life indoors. The home has been shown to be a major reservoir for indoor allergens. Change in the indoor environment through recent decades has resulted in increased home exposure that may contribute to the increased trend in prevalence and incidence of asthma in developed countries [2, 3].

Fungi can be found throughout the world. Fungal spores and other fungal particles are widely distributed in the outdoor and indoor environment. Increased fungal spore levels in a patient's environment have been associated with the presence of allergic symptoms and even asthma deaths [4]. Immunoglobulin E- mediated sensitivity to fungal allergens is common among patients with allergic rhinitis or allergic asthma [5]. It has been reported that up to 80% of subjects with confirmed asthma may have positive skin test reactions to one or more fungi [6]. Fungi frequently form the majority of suspended biologic particles in the air. The inhalation of fungus has been shown to induce airway symptoms and reduce pulmonary function in patients with sensitivity to fungi [7, 8]. The importance of fungal allergens in asthma has been well reviewed by Portnoy [9].

Fungal species in indoor and outdoor environments have been described for various regions [10-14]. Several common fungal species have been found in homes with asthmatic patients [2, 15, 16]. However, fungal species and airborne concentrations vary according to geographic location, season, sampling approach, humidity, and building characteristics [10, 11, 13, 14, 17]. Identification of the spectrum of fungal species in asthmatic homes

will provide information about potential intervention targets for promoting asthma care and improving the understanding of possible mechanisms involved in fungi associated asthma.

As allergic asthma patient visits are mostly observed seasonally, we explored airborne fungal species and concentrations in homes with asthmatic children mostly during fall and spring in comparison to homes with no asthmatic patients in winter cold and flu season using both spore counting and cultural approaches. This association was also examined while adjusting for seasonal factors and outdoor levels using multiple linear models.

METHODS

Study Design and Population This study was designed to examine common fungal species and compare their distribution and levels in asthmatic and non asthmatic homes. We recruited families with and without asthmatic children, obtained medical history from family members especially children, and collected air samples from inside and outside these homes.

The studied population includes families who participated in a home cleaning research project as described by Barnes et al previously [18]. Households contained at least three persons with at least one person between 2 and 17 years of age. The family must have lived in the same home, either rental or self-owned for at least 2 months. Families were excluded if an occupant stated they were allergic or sensitive to cleaning products, if the home was grossly contaminated with fungi, or if it was mechanically unsound or generally unsafe. Families were also excluded if air sampling was not completed due to various reasons.

Asthmatic families around the greater Kansas City area were identified from allergy clinic visits at the Children's Mercy Hospital (CMH). Each asthmatic home had at least one child with persistent asthma as defined by National Heart, Lung, and Blood Institute guidelines [19]. Homes in which there is no asthmatic child (non asthmatic homes) were

identified by asking asthmatic participants to refer a family in their neighborhood or from volunteers recruited within CMH.

The study was approved by the Children's Mercy Institutional Review Board. An initial health and wellness questionnaire for each proband and his or her eligible siblings was completed by the parent or guardian through a telephone interview and families were initially enrolled based upon on this information. An approved informed consent document was obtained and environmental surveys were conducted in a candidate home during the first visit to the home. A questionnaire, based on a standardized protocol [12], was completed to obtain background information such as age and type of building, use of the home in the past or environmental characteristics, age and type of heating, ventilation, and air conditioning system, resident's symptoms and medical history, environmental triggers, and home potential contamination sources [12]. For qualified homes, all health and wellness questionnaires for each proband and his or her eligible siblings were completed by the parent or guardian through a telephone call. Asthmatic homes were enrolled in the fall, summer and spring when their allergies might be worse and non asthmatic homes were recruited mainly during the winter cold and flu season. Two weeks after enrollment, the home was sampled according to standard protocols for examination of fungal species as described in the following.

Air sampling and estimation for fungal spores At each home, air sampling was conducted in four locations including the main living space (living/TV/family room/kitchen); bedroom of the enrolled child; the basement and outdoors separately using an Allergenco MK-3 as described previously [12, 20]. Since the structures were different in the studied apartments and houses, air samples in main living space were obtained in at least one location of the living room, TV room, family room and kitchen. Multiple samplings in the main living space were collected in some of the homes. Air samples for outdoor spore levels were collected at 3 feet above the ground and at distance with at least 10 feet from the house or apartment. The preferred location was on the side walk in the front side of the structure. The inlet velocity was 15L per minute for 10 minutes in each

location. The air samples collected on slides were observed at 400x for general spore content and then counted and identified at 1000x [12]. Total fungi and specific fungal genera were calculated as spore counts per cubic meter of air and rounded to the nearest integer. The average spore counts among the studied child's bedroom and main living spaces were calculated as the estimate of house fungal spore level. The home was defined as positive for spore detection if the fungal spore counts were greater than zero with regard to total or specific fungal species.

Air sampling and estimation for culturable airborne fungi At each home, air collections for culturable airborne fungi were taken in the main living space (living/TV/family room) directly onto malt extract agar (MEA) (Remel, Lenexa, KS, USA) using a BioStage Single-stage Viable Cascade Impactor (SKC Inc. Eighty Four, PA, USA) set to collect at 10 or 15 liters per minute for 20 minutes. MEA was prepared by using 12.75 g of malt Agar, 2.75g of maltose, 2.35 g of dextrin, 0.78 g of glycerol, 15.0 g of gelatin peptone in 1 liter of deionized water adjusted to pH 4.7. Fungal units were collected on a BioStage Single Stage Viable Spore collector. After 5 days samples were examined for growth of colonies and reported as estimates of fungal colony-forming units (CFUs) per cubic meter of air for total fungi and identifiable genera. No correction for multiple hits was applied. If fungal CFUs per cubic meter of air in a home were greater than zero, the home was defined as positive for detection of that specific culturable fungal taxa.

Statistical Analysis

Statistical analysis was completed using SAS 9.13. The frequency of positive fungal detection for either culturable fungi or fungal spore counts was calculated by dividing the number of positive homes with total homes in each category of families. Since total culturable fungal CFUs were relatively higher in April, May, Aug, Sept, Oct, and Nov than that in Jan, Feb, March, Jun, July, and Dec (see the supplementary figure 1), seasonal factor, a binary variable with high or low season, was generated for culturable airborne fungal data analysis. A Chi-Square test was generally applied for comparing frequency

between two groups and the Fisher exact test was applied if 50% of cells in the contingency table had expected counts less than 5. An odds ratio (OR) and its 95% confidence intervals for an association of positive fungal detection with asthma homes were calculated in a logistic regression analysis. To adjust for confounding factors, the adjusted OR value and its 95% confidence interval were calculated when the factor was considered as a covariant in the logistic regression model. For quantitative variables such as culturable fungal colonies and fungal spore counts, data normality in each category was examined. If the data was not normally distributed using Kolmogorov-Smirnov test, log transformation was conducted and normal distribution of log transformed data was further verified. A T test was used to compare average levels between two groups if data normality was confirmed. If data distribution was not normal, the Mann Whitney U test was applied to test the difference between two groups. An adjustment of confounding factors for quantitative traits was performed in a linear regression model when the factor was taken as a covariant. In correlation analysis between log transformed data of outdoor and indoor fungal spore counts, spearman correlation coefficient (r) was calculated.

RESULTS

Demographic features of the studied families

A total of 173 families were recruited into this study, including eighty-eight (asthmatic) families with at least one asthmatic child and eighty-five (non asthmatic) families without asthmatic children at home (table 1). The age for children with a completed medical history ranged from 2 to 18 years with an equal distribution of males and females in both sets of homes. About 76.5% of children from asthmatic homes reported nose/ ear/throat symptoms, 63% reported breathing problems and 43% reported skin problems. About 25% of asthmatic children had at least one emergency visit within the last year and 7.4% of them had at least one hospitalization. Fewer children from non asthmatic homes reported upper respiratory symptoms or skin problems.

Indoor fungal spore counts in asthmatic homes

A total of 169 families had indoor fungal spores present in the living room area and the room of the enrolled child (table 2). At least one fungal taxon was found in each of the homes. Eleven fungal spore taxa were detectable as listed in table 2. *Cladosporium*, ascospores and smuts/myxomycete spores existed in almost all homes with few exceptions regardless of asthmatic status. Basidiospores and *Alternaria* were also commonly found in about 50% of the homes. *Pithomyces*, *Epicoccum*, *Aspergillus_Penicillium* and *Curvularia* were detected in about 20% of homes. However, *Stachybotrys* and *Chaetomium* were rarely identified in these homes.

Basidiospores and *Epicoccum* were found with greater frequency in asthmatic homes than in non asthmatic homes. Their OR values were about 2-3 on average (table 2). Mean spore levels for both were significantly higher in positive asthmatic homes even after adjusting for the impact of outdoor spore counts as a potential confounding factor (table 3).

Although *Cladosporium*, ascospores, *Aspergillus/Penicillium*, and *Pithomyces* were not found in greater frequency in asthmatic homes when compared to non asthmatic homes, there were significantly higher levels of these fungal spores in the homes of families with asthmatic children (table 3). After adjusting for outdoor fungal spore counts for the same home, *Aspergillus/Penicillium* was still higher on average in asthmatic homes, but there was no difference for *Cladosporium*, ascospores and *Pithomyces*. Also average spore counts for *Aspergillus/Penicillium* were the highest among the fungal spores detected in the asthmatic homes.

Outdoor vs indoor fungal spores in asthmatic homes

In an attempt to clarify the sources of fungal exposure for asthmatic children, we examined the correlation of outdoor vs. indoor fungal spores. A significant correlation of indoor and outdoor spore counts existed in the fungal species. The most frequently identified fungi such as ascospores and *Cladosporium* were found simultaneously in both

indoor and outdoor collections and correlated well (ascospores: $r=0.45$, $p<0.0001$; *Cladosporium*: $r=0.36$, $p<0.0001$ and total fungal spores: $r=0.41$, $p<0.0001$) suggesting that indoor spore levels may be impacted by outdoor levels. The fungal taxa such as basidiospores, *Pithomyces*, *Aspergillus/Penicillium* and *Epicoccum* were found in indoor collections but not in outdoor collections for some homes, or in outdoor but not in indoor for other homes (Figure 1).

Viable fungal species in asthmatic homes

As viable fungi may colonize the human body through inhalation, and damage airways by the production of toxins, proteases, enzymes and volatile organic compounds, the culturable fungal species were further analyzed. The results showed that 165 families (97%) had at least one fungal species detectable in air samples collected from the main living space (living/TV/family room) (table 4). Among them 18 species were found in either 84 asthmatic or 81 non asthmatic homes, but *Bispora*, *Botrytis*, *Cercospora*, *Oidium*, *Puccinia*, *Spegazzinia*, *Stachybotrys*, *Stemphylium*, *Tetraploa* and *Torula* were not detected in any home. The observation that culturable *Cladosporium*, *Penicillium*, *Alternaria*, *Aspergillus*, *Epicoccum*, *Curvularia* and *Pithomyces* were the most common fungal taxa detected in both types of homes confirmed our findings from non-viable fungal spore counts. Furthermore, more fungal species including *Fusarium*, *Rhizopus*, *Geotrichum*, *Ulocladium*, *Acremonium*, *Nigrospora*, *Sordaria*, *Paecilomyces*, and *Chaetomium* were identified in both asthmatic and non asthmatic homes although they were rarely found.

In asthmatic homes, the most frequently detected culturable fungal species were *Cladosporium*, *Penicillium*, *Alternaria*, *Aspergillus*, *Epicoccum*, and *Curvularia*. Culturable *Penicillium*, *Curvularia* and *Fusarium* were found more often in homes of asthmatics. The significant difference in the finding of *Penicillium* seems not to be influenced by seasonal variation as the p value was still less than 0.05 after adjustment for seasonal factors (table 4).

The average culturable colony levels of *Cladosporium*, unknown and total fungal spores were significantly higher in asthmatic homes (table 5). The average culturable CFUs of *Penicillium* and *Alternaria* were higher in asthmatic homes at close to threshold significance level. The adjustment of seasonal factors resulted in no significance for average CFUs of *Cladosporium*, *Penicillium* and *Alternaria* between asthmatic and non asthmatic homes. However, the average culturable colony levels of unknown and total fungi were still significantly higher in asthmatic homes even after adjusting the possible confounding effect from seasonal factors.

Seven culturable species including *Geotrichum*, *Ulocladium*, *Acremonium*, *Bipolaris/Drechslera*, *Paecilomyces* and *Chaetomium* were detectable in several asthmatic homes, but not detectable in non asthmatic homes (table 4). And, *Sordaria* was detected only in non asthmatic homes (table 4).

DISCUSSION

Fungal growth is impacted by temperature, humidity, building materials and other factors. These may account for the variation of fungal species and count levels in different geographic locations, seasons, and building characteristics in the literature [10, 11, 13, 14, 17]. This study recruited families located in various regions including rural (5%), urban core (40%) and suburban (55%) in the greater Kansas City area. The homes varied from apartment to single family home. All homes in the study were in good condition and none had a chronic problem with water intrusion, leaking plumbing or fungal contamination issues. The homes had carpeting on ~50% of the flooring area with 11% of homes containing no wall to wall carpet. The homes were all air-conditioned and centrally heated by gas forced air. Indoor temperatures were maintained around 22°C and 50% relative humidity. Outdoor temperatures during the study period ranged from near 38°C to below freezing [18]. These are typical residential home conditions in the Middle West region of the United States. Air sampling ranged throughout all seasons in respect to growth of fungi.

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In our recruited homes, at least one fungal species was identified from the air samples collected in each home indicating the widespread existence of fungi in typical resident homes in this area. A total of twenty-one fungal species were identified through either viable fungal culture or fungal spore counting. Consistent with our previous and other reports [11, 12], the predominant species were *Cladosporium* (100% spore counts vs 87.1% culturable), ascospores (98% spore counts), smuts/myxomycetes (93.5% spore counts). The more common species include *Alternaria* (51.5% spore counts vs. 50% culturable), basidiospores (59.2% spore counts), *Penicillium* (19.5% spore counts vs. 66.5% culturable), *Aspergillus* (19.5% spore counts vs. 37.7% culturable), *Curvularia* (13.6% spore counts vs. 10.6% culturable), *Epicoccum* (24.3% spore vs. 15.9% culturable), *Pithomyces* (25.4% spore counts vs. 5.3% culturable), *Fusarium* (5.3% culturable), *Rhizopus* (6.5% culturable). Rare species found were *Sordaria* (3.5% culturable), *Stachybotrys* (3.0% spore counts), *Chaetomium* (2.4% spore counts vs. 0.6% culturable), *Geotrichum* (2.4% culturable), *Nigrospora* (2.4% culturable), *Ulocladium* (2.3% culturable), *Acremonium* (1.8% culturable), *Bipolaris/Drechsleria* (0.6% culturable), *Paecilomyces* (0.6% culturable). Compared to our previous report, the rate of moisture indicator fungi including *Chaetomium*, *Stachybotrys* and *Ulocladium* was low. This may be because homes with water leakage were excluded from this study. However, since some *Stachybotrys* and *Chaetomium* spores were found in these homes, there may be hidden or subtle water damage. Therefore even with a negative history of water intrusion, a home evaluator schooled in fungal ecology can often identify water problems.

The asthmatic families recruited in the current study were identified from the greater Kansas City area, a typical Middle West city in US because they had at least one asthmatic child in their homes. Most of the asthmatic children had clinical symptoms as shown in table 1. In asthmatic children recruited for a healthy home project conducted at CMH, the allergic sensitization rate for at least one of eight common allergens including cat, dog, rodent, aspergillus, penicillium, alternaria, cladosporium, dust mite and cockroach was 57.5% and for alternaria, aspergillus, cladosporium and penicillium was 27.5%, 20%,

17.5% and 15% respectively (data not published). We expect the asthmatic children had a similar allergic sensitization rate because they were from the same population. As asthma and allergic clinic visits are mostly observed during late spring and fall, asthma related fungal species and spore levels during these seasons are expected to be higher than during the winter flu seasons. The recruitment of asthmatic homes mostly during late spring and fall and non asthmatic homes during flu season was expected to identify more related fungal species with asthma. As one of the most identified spore types, ascospores derive from several species such as *Leptosphaeria*, *Nectria* and other unidentified fungi classified as Ascomycota [21]. Ascospores were identified inside all homes. The spore level was significantly higher in asthmatic homes. A significant correlation of outdoor and indoor ascospores was found in almost every positive home. After adjustment with outdoor levels, no significance was observed between asthmatic and non asthmatic homes. This indicates that higher levels of ascospores in asthmatic homes may be due to the seasonal change and are mainly impacted by the outdoor level rather than the difference between asthmatic and non asthmatic home indoor environments. Smuts/Myxomycetes showed no difference between asthmatic and non asthmatic homes in this study although they were widely distributed in both asthmatic and non asthmatic homes. The significance of these fungi remains to be explored in the future although human sensitivity to a few obtainable spore types has been demonstrated especially in Great Britain [22].

Cladosporium, *Penicillium*, *Aspergillus*, basidiospores and *Curvularia* are the fungi most frequently associated with asthma [9, 23]. The differences in their distribution between asthmatic and non asthmatic homes were observed in this study also. The importance of *Cladosporium* in asthma has been recognized in the literature. So far, 14 allergens have been identified from two species *Cladosporium herbarum* and *Cladosporium cladosporioides* [23]. *Cladosporium* is believed to be a predominant outdoor fungal species outnumbering all other outdoor bioaerosols [24]. Our results indicated that its spores were found in all homes and its level in asthmatic homes was significantly higher. The correlation of outdoor and indoor *Cladosporium* spore levels and the evidence that the significantly different spore levels diminished after adjustment of

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outdoor levels confirmed that indoor spores were really impacted by outdoor spore counts. The results of culturable *Cladosporium* confirmed the phenomenon observed suggesting that the tightness between outdoor and indoor may influence the indoor *Cladosporium* levels. Meanwhile, there was detectable *Cladosporium* inside a few houses where no spores were detected outdoors. This suggested that *Cladosporium* conidia could possibly grow inside homes or persist indoors for a longer time after penetration into the building, and account for a certain portion of *Cladosporium* exposure. The significant difference for culturable fungal measurement between asthmatic and non asthmatic homes suggested that the living *Cladosporium* inside the homes may play an important role in exacerbating asthmatic and allergic symptoms during late spring and fall seasons.

Penicillium and *Aspergillus* have been reported to be dominant indoor fungi in various buildings especially in sick dwellings [25-27]. Our results indicated that *Aspergillus/Penicillium* was only found in about 20% of asthmatic homes and culturable *Aspergillus* colonies were found in about 34% of asthmatic homes. As indoor humidity is an important factor for the growth of xerophilic fungi such as *Penicillium* and *Aspergillus*, the relatively low spore detection rate for *Penicillium* and *Aspergillus* may reflect relative better conditions in the studied homes. Our results showed that average *Aspergillus/Penicillium* spore concentrations were the highest in positive asthmatic homes. Culturable *Penicillium* colonies were among the most frequently identified species especially in asthmatic homes more than in non asthmatic homes. This did not vary from season to season. Meanwhile, with respect to the source, *Aspergillus/Penicillium* spores were found outdoors in a few homes, but they were often found indoors. This suggests a ecological difference between the groups of homes. *Aspergillus/Penicillium* spores do not typically dominate the air-spora in buildings free of dampness or water damage. The relation of damp indoor environments to respiratory disease has been extensively documented [28]. The significant higher spore counts of *Aspergillus/Penicillium* in asthmatic homes may not be impacted by outdoor spore counts. These consistently support that home *Penicillium* and *Aspergillus* are important indoor fungi for asthmatic patients. Taking into consideration the fact that multiple allergens have been identified and that the

association between allergic sensitization and asthmatic phenotypes has been reported [29, 30], remediation efforts directed against *Aspergillus/Penicillium* in the indoor environment will be expected to benefit asthmatic care.

Basidiospores develop on specialized tissues called basidia, and are discharged from diverse forms of basidiomycetes such as puffballs, mushrooms, rust and smuts [21]. In temperate zones, seasonal peaks of basidiospores are observed in spring and autumn [23]. Increased epidemics of asthma and hospitalizations have been associated with high basidiospore counts [31, 32]. Our results consistently confirmed that basidiospores were significantly higher in asthmatic homes. The significant higher detection rate of basidiospores diminished after adjustment for outdoor basidiospore counts. This may partly be because basidiospores in asthmatic homes were mostly counted during late spring and fall seasons. The significant correlation of outdoor and indoor basidiospore spores further provides evidence for the contribution of outdoor to indoor concentrations. The tightness of the houses is expected to influence the indoor basidiospore counts. Meanwhile, in some homes basidiospores were detected in indoor but not in outdoor collections. This can explain the observation that basidiospore spore concentrations were still significantly higher in asthmatic homes even after adjustment for outdoor influence. Although basidiospores are not expected to grow in indoor environments, there could be many reasons for indoor outdoor differences including sampling bias and a continued presence indoors after a short release period [33].

Curvularia is a fungal species found in the Southern and Midwestern United States. Protein allergens from *Curvularia* have been reported. The allergic components such as Cur l 2 (enolase), Cur l GST, Cur l SOD, Cur l 1 (serine protease) and Cur l Trx showed cross reactivity with fungal allergens from other Phyla [23]. Sensitization to *Curvularia* has been found to be associated with increased numbers of asthma exacerbations requiring hospitalization [34]. *Fusarium* is an important spoilage organism and plant pathogen. Mycotoxin and allergens generated from *Fusarium* extracts may be related to asthmatic patients [23, 35]. The cross reactivity of its allergic components such as Fus c 1 and Fus c

2 with other fungal allergens has been identified. Our results indicated that culturable *Curvularia* and *Fusarium* were found in more asthmatic homes than that in non asthmatic homes although adjusting for seasonal factors diminished the statistical significance. The significance of this finding in relation to asthma remains to be illustrated.

As our recruited houses are located in the Midwest US amid agricultural and grassland areas, it is not surprised that *Epicoccum* spores are widespread and could be found in more than 30% of asthmatic homes in our study [21]. The allergens in *Epicoccum* such as Epi p GST1 and Epi p 1 (serine protease) have been identified and showed cross-reactivity with other allergens [23]. Both *Epicoccum* frequency and concentrations were found to be increased in asthmatic homes. These differences seem not to be impacted by outdoor sources significantly. Significantly higher *Pithomyces* spores were also found in asthmatic homes. The outdoor air seems to be a major source of *Pithomyces* compared to the indoor environment. To our knowledge, the association of *Pithomyces* and *Epicoccum* with asthma has rarely been documented in the literature although often suspected. The cross-reactivity of their allergic components with other common fungal or non fungal allergens components may play an important role in the development of asthma. We also noticed that there were significantly higher culturable unknown fungal colonies in asthmatic homes. It can be expected that additional asthmatic associated fungal species may be identified in the future.

The comparison between asthmatic and non asthmatic homes can help to identify environmental problems unique to asthmatic patients as intended in this study. Significant differences in either detection rate or concentration of fungal species existed between asthmatic homes and non asthmatic homes. As many of them are known allergen sources, identifying and controlling these fungal species may potentially improve asthma care. To understand why there are differences in fungal species between asthmatic and non asthmatic homes in addition to seasonal factors is important and will help to benefit this purpose. However, it is hard to draw a conclusion about whether this environmental exposure can be linked to causes of asthma incidence or asthma exacerbation from the

present study. As the population is derived from a cleaning product research project, some homes with grossly contaminated fungi and unsound and unsafe houses were excluded. It is expected that more or different fungal species and higher levels of spores may be experienced by asthmatic children in these poorly maintained residences. In addition, culture technique was used to identify the fungal species which grow on specific agar medium in this study because it is considered as a reliable and economical approach up to now. However, it would be expected that many yeasts and other fungal species with more rigorous nutrient requirements may have been completely overlooked. For fungal species with no difference between asthmatic and non asthmatic homes, it does not mean that we should ignore them in improving asthma home care. *Alternaria* is a significant fungal species which has been associated with various asthmatic phenotypes [9]. The major allergens of *Alternaria* have been reported to cross-react with other fungal allergens especially in other members of the Pleosporaceae family such as *Stemphylium*, *Ulocladium*, and *Curvularia* [23, 36]. The negative finding from this study may suggest that this fungal species is found in every kind of home and season [37]. As its importance in asthma and allergic diseases has been verified in previous studies, it is reasonable to pay attention to its existence in home remediation. To understand why there are differences in fungal distribution between asthmatic and non asthmatic homes and confirm whether these fungal species can cause asthma, asthma symptoms and asthma exacerbation is our focus for further research.

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Figure 1. Correlation of Indoor and outdoor fungal spore counts in asthmatic and non asthmatic homes. A. Basidiospores. B. *Aspergillus/Penicillium*. C. *Epicoccum*. D. *Pithomyces*. E. *Curvularia*. R: spearman correlation coefficient. Blue dot: non asthmatic homes. Red dot: asthmatic homes.

Table 1. Demographic features of the participated asthmatic and non asthmatic families

Variables	Asthmatic % (N)	Non-Asthmatic % (N)	P value
Age	8.91 $\pm$ 4.15 (2–17.5)	6.98 $\pm$ 3.84 (2–17.3)	0.0002
Male	49.6 (58)	47.2 (59)	0.90
Present with nose/ear/throat problems	76.5 (88)	3.1 (4)	<0.0001
Present with respiratory problems	63.2 (72)	0	<0.0001
Present with skin problems	43.0 (49)	7.0 (9)	<0.0001
At Least one emergency visit last Year	25.0 (27)	0	<0.0001
At least one hospitalization last year	7.4 (8)	0	0.002
Total children	115	128	
Total families	88	85	

The frequency (%), number of children (N), mean and standard deviation of age in each category are listed in the table.

Table 2. Frequency of fungal spore detection in air samples from subject and living room in asthmatic and non asthmatic homes

Fungus	All Positive Rate (%) (N)	Asthmatic Positive Rate (%)(N)	Non-Asthmatic Positive Rate (%) (N)	P value	OR	Adjusted OR*	Adjusted P value*
Cladosporium	100 (169)	100.0 (88)	100.0 (81)	NA	NA	NA	NA
Ascospores	98.2 (166)	100.0 (88)	96.3 (78)	0.11	NA	NA	NA
Smuts/Myxomycetes	93.5 (158)	92.1 (81)	95.1 (77)	0.43	0.60 (0.17, 2.14)	0.61 (0.17, 2.17)	0.44
Basidiospores	59.2 (100)	71.6 (63)	45.7 (37)	0.0006	3.0 (1.59, 5.67)	1.71 (0.83, 3.53)	0.15
Alternaria	51.5 (87)	58.0 (51)	44.4 (36)	0.08	1.72 (0.94, 3.17)	1.33 (0.70, 2.53)	0.39
Pithomyces	25.4 (43)	30.7 (27)	19.8 (16)	0.10	1.80 (0.88, 3.66)	1.41 (0.67, 2.98)	0.37
Epicoccum	24.3 (41)	31.8 (28)	16.1 (13)	0.02	2.44 (1.16, 5.13)	2.27 (1.06, 4.83)	0.03
Aspergillus/Penicillium	19.5 (33)	18.2 (16)	21.0 (17)	0.65	0.84 (0.39, 1.79)	0.88(0.41, 1.90)	0.75
Curvularia	13.6 (23)	14.8 (13)	12.4 (10)	0.65	1.23 (0.51, 2.99)	0.94 (0.36, 2.43)	0.89
Stachybotrys	3.0 (5)	2.3 (2)	3.3 (3)	0.58	0.61 (0.10, 3.71)	0.91 (0.13, 6.59)	0.92
Chaetomium	2.4 (4)	2.3 (2)	2.5 (2)	1.0	0.92 (0.13, 6.68)	0.90 (0.12, 6.51)	0.91
Total fungi spores	100 (169)	100.0 (88)	100.0 (81)	NA	NA	NA	NA

The frequency (%) and number of homes (N) in each category, OR (95% confidence interval) and p value for the comparison are listed in the table. * The adjusted OR and p value are also listed when outdoor fungal spore was considered as a confounding factor. NA: Not applicable.

Table 3. The average fungal spore counts in air samples from living and subject room in asthmatic and non asthmatic homes

Fungi	Asthmatic				Non-Asthmatic				P value	Adjusted P value*
	N	Mean $\pm$ SD (ln)	Min	Max	N	Mean $\pm$ SD (ln)	Min	Max		
Aspergillus/Penicillium	16	5.89 $\pm$ 1.63	33	8875.5	17	4.74 $\pm$ 1.88	7.5	4609	0.07	<0.04
Cladosporium	88	5.61 $\pm$ 1.32	15	5718	81	5.06 $\pm$ 0.97	22	2262.5	0.01	0.17
Ascospores	88	5.04 $\pm$ 1.33	11.5	4634	78	4.25 $\pm$ 0.99	7.5	875.5	0.0001	0.27
Basidiospores	63	3.65 $\pm$ 1.12	7.5	1579.5	37	3.06 $\pm$ 0.83	4.5	171.5	0.01	<0.05
Smuts/Myxomycetes	81	3.62 $\pm$ 1.13	4.5	641	77	3.73 $\pm$ 1.05	7.5	492	0.52	0.33
Alternaria	51	3.0 $\pm$ 0.82	4.5	118	36	2.95 $\pm$ 0.76	4.5	65	0.75	0.89
Curvularia	13	2.67 $\pm$ 1.04	4.5	171	10	2.12 $\pm$ 0.60	4.5	22.5	0.16	0.50
Epicoccum	28	2.44 $\pm$ 0.80	4.5	139.5	13	1.95 $\pm$ 0.43	4.5	22.5	<0.05	<0.05
Phthomyces	27	2.37 $\pm$ 0.66	4.5	54.5	16	1.87 $\pm$ 0.36	4.5	11.5	<0.01	0.09
Chaetomium	2	3.19 $\pm$ 1.66	7.5	78.5	2	1.76 $\pm$ 0.36	4.5	7.5	0.36	NA
Stachybotrys	2	4.58 $\pm$ 1.95	24.5	385	3	3.17 $\pm$ 1.23	7.5	86.5	0.38	0.30
Total fungi spores	88	6.45 $\pm$ 1.26	53.5	15787.5	81	5.83 $\pm$ 1.02	56.5	5533	<0.01	0.26

The mean and standard deviation (ln value), minimum and maximum of average fungal spore counts between subject and main living space in positive asthmatic or non-asthmatic homes are listed. *Outdoor fungal spore counts were adjusted in a multiple linear regression model.

Table 4. Frequency of viable fungi in air samples identified in asthmatic and non asthmatic homes

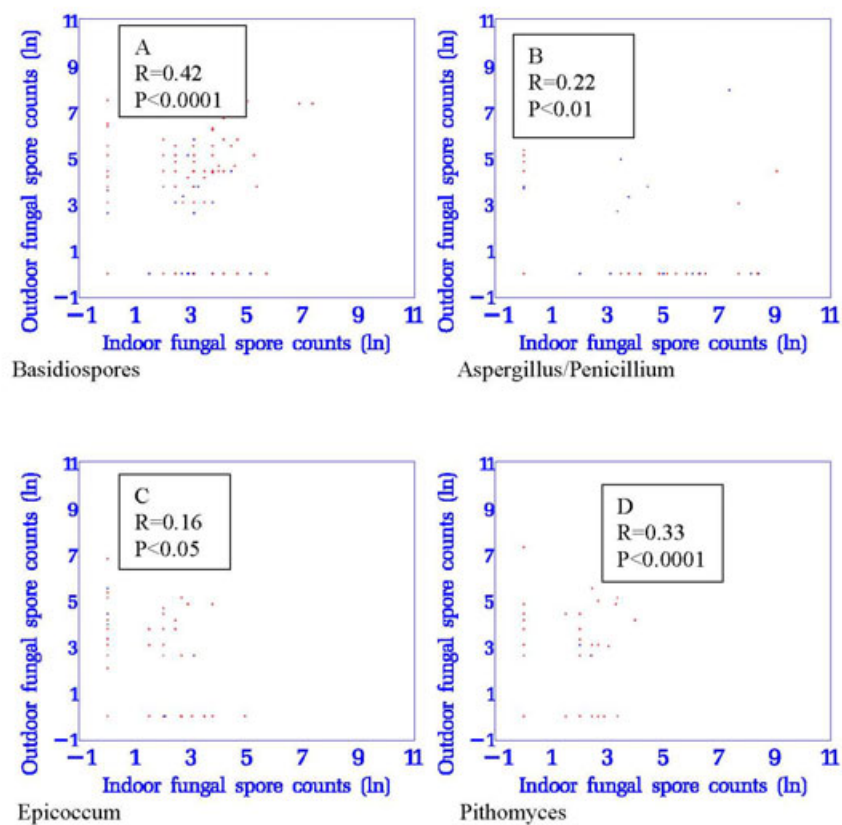
Allergens	Whole group	Asthmatic	Non-Asthmatic	P value	OR	Adjusted OR*	Adjusted P value*
	Positive Rate (%) (N)	Positive Rate (%) (N)	Positive Rate (%) (N)				
Cladosporium	87.1 (148)	86.1 (74)	88.1 (74)	0.69	0.83 (0.34, 2.05)	0.75 (0.29, 1.92)	0.55
Penicillium	66.5 (113)	75.6 (65)	57.1 (48)	<0.02	2.32 (1.21, 4.47)	2.38 (1.19, 4.76)	<0.02
Alternaria	50.0 (85)	51.2 (44)	48.8 (41)	0.76	1.10 (0.60, 2.01)	0.90 (0.47, 1.72)	0.75
Aspergillus	37.7 (64)	33.7 (29)	41.7 (35)	0.29	0.71 (0.38, 1.33)	0.84 (0.43, 1.61)	0.59
Epicoecum	15.9 (27)	17.4 (15)	14.3 (12)	0.57	1.27 (0.55, 2.90)	1.15 (0.48, 2.77)	0.75
Curvularia	10.6 (18)	16.3 (14)	4.8 (4)	<0.02	3.89 (1.22, 12.36)	3.15 (0.96, 10.38)	0.06
Fusarium	5.3 (9)	9.3 (8)	1.2 (1)	<0.03	8.51 (1.04, 69.64)	7.25 (0.85, 61.70)	0.07
Pithomyces	5.3 (9)	8.1 (7)	2.4 (2)	0.17	3.63 (0.73, 18.02)	2.11 (0.41, 10.80)	0.37
Rhizopus	6.5 (11)	7.0 (6)	5.9 (5)	0.79	1.18 (0.35, 4.04)	0.84 (0.23, 3.00)	0.78
Geotrichum	2.4 (4)	4.7 (4)	0 (0)	0.12	NA		
Ulocladium	2.3 (4)	4.7 (4)	0 (0)	0.12	NA		
Acremonium	1.8 (3)	3.5 (3)	0 (0)	0.25	NA		
Nigrospora	2.4 (4)	1.2 (1)	3.6 (3)	0.37	0.32 (0.03, 3.12)	0.44 (0.04, 4.67)	0.49
Bipolaris/Drechsleria	0.6 (1)	1.2 (1)	0 (0)	1.0	NA		
Paecilomyces	0.6 (1)	1.2 (1)	0 (0)	1.0	NA		
Chaetomium	0.6 (1)	1.2 (1)	0 (0)	1.0	NA		
Sordaria	3.5 (6)	0 (0)	7.1 (6)	0.01	NA		
Unknown	51.8 (88)	53.5 (46)	50.0 (42)	0.65	1.15 (0.63, 2.10)	1.21 (0.64, 2.29)	0.55
Total fungi	97.06 (165)	97.7 (84)	96.5 (81)	0.68	1.56 (0.25, 9.55)	1.71 (0.26, 11.36)	0.58

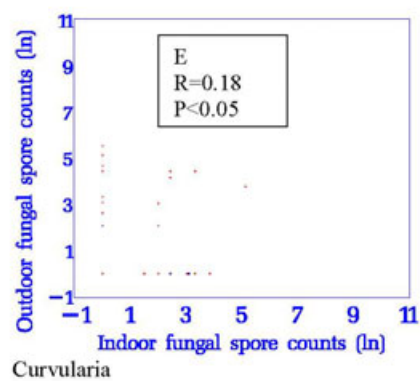
The frequency (%) and number of homes (N) in each category, OR (95% confidence interval) and p value for the comparison are listed in the table. *The adjusted OR and p value are also listed when seasonal factor was considered as a confounding factor. NA: Not applicable.

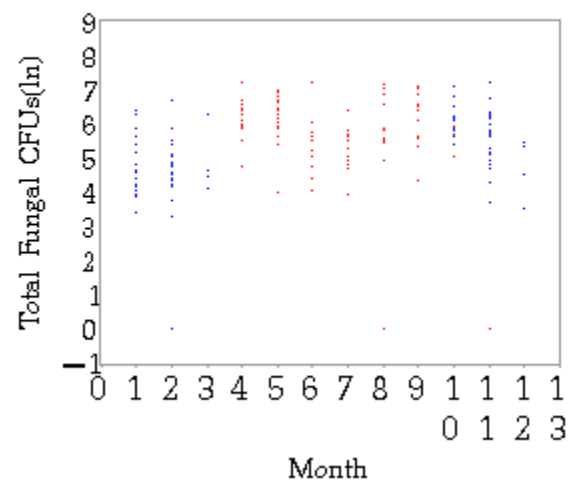
Table 5. Viable fungal colony level in air samples collected from asthmatic and non asthmatic homes

Fungi	Asthmatic				Non-Asthmatic				P value	Adjusted P value*
	N	Mean $\pm$ SD	Min	Max	N	Mean $\pm$ SD	Min	Max		
Cladosporium	74	5.18 $\pm$ 1.21	11	1292	74	4.43 $\pm$ 1.03	10	1167	<0.0001	0.07
Penicillium	65	4.12 $\pm$ 1.15	3	1163	48	3.72 $\pm$ 1.31	5	610	0.09	0.34
Alternaria	44	3.99 $\pm$ 1.02	5	410	41	3.60 $\pm$ 0.93	3	245	0.07	0.36
Epicoecum	15	3.63 $\pm$ 1.02	7	356	12	3.62 $\pm$ 0.91	9	177	0.98	0.84
Aspergillus	29	3.62 $\pm$ 0.77	11	310	35	3.33 $\pm$ 1.20	3	333	0.24	0.59
Fusarium	8	3.62 $\pm$ 1.21	11	265	1	4.07	57	57	0.56	0.91
Phthomyces	7	3.60 $\pm$ 0.41	20	59	2	3.88 $\pm$ 1.32	18	122	0.82	0.59
Curvularia	14	3.42 $\pm$ 0.55	11	76	4	3.22 $\pm$ 0.71	9	50	0.56	0.57
Rhizopus	6	3.42 $\pm$ 0.58	14	57	5	4.69 $\pm$ 0.96	43	480	0.02	0.09
Geotrichum	4	3.41 $\pm$ 0.79	11	82	0	0	NA	NA	<0.01	NA
Acremonium	3	3.32 $\pm$ 0.67	15	57	0	0	NA	NA	<0.02	NA
Bipolaris/Drechsleria	1	3.41	29	29	0	0	NA	NA	NA	NA
Paecilomyces	1	3.26	26	26	0	0	NA	NA	NA	NA
Puccinia	1	3.26	26	26	0	0	NA	NA	NA	NA
Ulocladium	4	3.06 $\pm$ 0.33	15	28	0	0	NA	NA	<0.001	NA
Nigrospora	1	1.90	6	6	3	3.63 $\pm$ 0.88	13	67	0.23	0.45
Chaetomium	1	2.96	18	18	0	0	NA	NA	NA	NA
Sordaria	0	0	0	0	6	3.22 $\pm$ 1.45	5	333	<0.01	NA
Unknown	46	3.70 $\pm$ 0.98	3	286	42	3.06 $\pm$ 0.78	5	236	<0.01	<0.01
Total	84	5.92 $\pm$ 0.84	50	1333	81	5.19 $\pm$ 0.95	27	1333	<0.0001	0.001

The mean and standard deviation (In value), minimum and maximum of viable fungal CFUs in positive asthmatic or non asthmatic homes are listed. *Seasonal factor was adjusted in a multiple regression model.







Supplementary figure 1. Total culturable fungal CFUs in asthma and non asthmatic homes by sampling months. Red dots for asthmatic homes and blue dots for non asthmatic homes.