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**Isolation of *Aspergillus fumigatus* from sputum is associated with elevated airborne levels in homes of patients with asthma**

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## Abstract

Indoor bioaerosols, such as mold spores, have been associated with respiratory symptoms in patients with asthma; however, dose-response relationships and guidelines on acceptable levels are lacking. Furthermore, a causal link between mold exposure and respiratory infections or asthma remains to be established. The aim of this study was to determine indoor concentrations of *Aspergillus fumigatus* and a subset of clinically relevant fungi in homes of people with asthma, in relation to markers of airways colonization and sensitization. Air and dust samples were collected from the living room of 58 properties. Fungal concentrations were quantified using mold-specific quantitative PCR and compared with traditional microscopic analysis of air samples. Isolation of *A. fumigatus* from sputum was associated with higher airborne concentrations of the fungus in patient homes ( $P = 0.04$ ), and a similar trend was shown with *Aspergillus/Penicillium*-type concentrations analyzed by microscopy ( $P = 0.058$ ). No association was found between airborne levels of *A. fumigatus* and sensitization to this fungus, or dustborne levels of *A. fumigatus* and either isolation from sputum or sensitization. The results of this study suggest that the home environment should be considered as a potential source of fungal exposure and elevated home levels may predispose people with asthma to airways colonization.

**Key words:** Air, dust, airways colonization, fungal sensitization, mold-specific quantitative PCR (MSQPCR), residential properties

## Practical Implications

Increased airborne mold concentrations in the home environment may lead to increased risk of airways colonization by fungi, suggesting that environmental exposure to fungi should be considered in the medical evaluation of asthma patients. No significant correlations were found between fungal concentrations in air and dust samples, suggesting that both airborne

and dustborne concentrations should be considered in assessments of fungal contamination of buildings.

## Introduction

It is recommended that optimal management of asthma should include identification and minimization of environmental exposures to irritants and allergens, including fungi (National Heart, Lung and Blood Institute, 2007). Fungal contamination of damp properties and associations with health have been widely reported; however, fungi such as *Aspergillus* and *Penicillium* are also common contaminants of non-complaint properties, (Ren et al., 2001; Fairs et al., 2010b), and the identification and eradication of fungal contamination may be of benefit to fungal-sensitized asthmatics.

Traditional analysis of fungal contamination of properties typically utilizes fungal culture from air or dust samples, or standard microscopy of air samples. Quantification by culture-based methods is susceptible to growth media bias, short sampling times and culture only of viable spores. Traditional microscopy methods enable longer duration of sampling and time-dependent analyses, without the bias of viability, but prevent discrimination between *Aspergillus* and *Penicillium* conidia, which are grouped together as 'Asp/Pen-type'.

Furthermore, spores from a number of other fungal genera including *Paecilomyces* and *Wallemia* are morphologically similar to those from the *Aspergillus* and *Penicillium* genera and may be included in analyses. An alternative method is mold-specific quantitative PCR (MSQPCR) which enables species-specific quantification of *Aspergillus* and *Penicillium* species with no limits on sample duration, or bias of viability. MSQPCR can be applied to both air and dust samples, enabling detection of higher levels and greater diversity of fungal contamination than traditional culture-based methods (Vesper et al., 2006). This technique

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has been used to generate environmental relative moldiness index values (ERMI, the ratio of fungi indicative of water-damage or visible mold contamination to common indoor fungi within properties), and high ERMI values from home dust samples have been associated with increased risk of asthma in children aged seven (Reponen et al., 2011). Using more traditional approaches, increased levels of viable *Aspergillus* and *Cladosporium* in house dust have been associated with allergic sensitization in children (Jacob et al., 2002); increased airborne home exposure to *Aspergillus* has been associated with asthma in children without a family history of the disease (Jones et al., 2011); and dustborne occupational exposure to viable fungi has been associated with new onset asthma, post-building occupancy in adults (Park et al., 2008). Remediation of moisture damage in properties has been associated with an improvement of asthma symptoms in children (Kercsmar et al., 2006) and a meta-analysis of the literature has shown that mold and home dampness is associated with a 30-50% increase in asthma and a variety of respiratory symptoms (Fisk et al., 2007). A recent epidemiological review (Mendell et al., 2011) concluded that whilst indoor mold and home dampness have been consistently associated with asthma development and exacerbations, allergy and respiratory symptoms, it is still not possible to describe dose-response relationships between microbial exposure and respiratory disease, to attribute causality, or to define safe levels of mold and damp-related exposures. Future studies recommended include efforts to determine the causality of exposure to mold or damp and respiratory infections or asthma (Mendell et al., 2011).

The ubiquitous nature of fungi means that exposure is inevitable, and in patients with lung disease such as asthma and cystic fibrosis, germination of inhaled fungi (predominantly *Aspergillus fumigatus*) within the bronchial tree can lead to airways colonization. *A. fumigatus* is an opportunistic fungal pathogen with both outdoor and indoor sources, growing

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outdoors in compost piles, and with sources indoors particularly in kitchens, bathrooms and cellars, and on substrates such as insulation material, soil of potted plants and in used pillows (Summerbell et al., 1992, Woodcock et al., 2006, Nilsson et al., 2004). The fungus has also been infrequently isolated from water-damaged floor materials and concrete (Andersen et al., 2011). Outdoor sources of *A. fumigatus* can reach high levels, particularly around compost sites, due to its fundamental role in degradation of organic matter (Fischer et al., 2008, Taha et al., 2006).

Sputum samples are frequently used in microbiological investigations of respiratory infections. We have shown previously that a focused approach towards detection of fungi in sputum significantly increases culture rates in comparison to standard procedures used in routine National Health Service clinical laboratories in the UK (Pashley et al., 2011).

Isolation of *A. fumigatus* from sputum has been associated with specific sensitization to the fungus, with 63% of *A. fumigatus*-IgE sensitized asthma patients being sputum culture positive for *A. fumigatus*, indicating airways colonization, in comparison to 31% of non-sensitized asthmatics and 7% of healthy subjects (Fairs et al., 2010a). *A. fumigatus* IgE-sensitized asthmatics had reduced lung function in comparison to non-sensitized asthma patients (Fairs et al., 2010a) and isolation of any filamentous fungi from sputum (predominantly *Aspergillus* and *Penicillium* species) has also been associated with reduced lung function in asthma (Agbetile et al., 2012), linking airways colonization by fungi with severity of the disease.

To our knowledge, there have been no studies to date relating home exposure to fungi with airway colonization or sensitization to *A. fumigatus*. Therefore, airborne and dustborne concentrations of a subset of fungi isolated from sputum, focusing primarily on *A. fumigatus*,

were used as a marker of home exposure and investigated for associations with measures of airways colonization and fungal sensitization.

## Methods

### Study cohort

The study participants comprised 58 adult asthma patients recruited consecutively from the difficult asthma clinic at Glenfield hospital, Leicester (UK); predominantly from a study to characterize *A. fumigatus*-associated asthma (Fairs et al., 2010a) and was approved by the Leicestershire and Rutland ethics committee. All subjects gave written, informed consent, had a physician diagnosis of asthma and underwent clinical assessment, including pulmonary function testing and methacholine challenge as described previously (Fairs et al., 2010a).

### Allergy testing

Atopy was assessed using routine skin prick tests to common aeroallergens including grass pollen, dog and cat fur, and *Dermatophagoides pteronyssinus*, and included an extended fungal panel of: *A. fumigatus*, *Alternaria alternata*, *Botrytis cinerea*, *Cladosporium herbarum* and *Penicillium chrysogenum* (Alk-Abello, Hørsholm, Denmark). Total IgE, *A. fumigatus*-IgE and *A. fumigatus*-IgG levels were measured using the UniCAP 250 system (Pharmacia, Milton Keynes, UK). Asthma patients were divided into groups according to *A. fumigatus* sensitization as previously described (Fairs et al., 2010a) : 1) *A. fumigatus*-IgE sensitized as defined by a positive skin prick test to *A. fumigatus* with wheal  $\geq 3$ mm or *A. fumigatus*-IgE  $>0.35$  kU/L; 2) IgG-only sensitized with *A. fumigatus*-IgG  $>40$  mg/L; and 3) non-sensitized.

### Collection of samples

Spontaneous or induced sputum samples were collected on a single, stable visit between August 2007 and November 2010. Sputum induction was performed using standard

techniques (Brightling et al., 2000, Pizzichini et al., 1996, Pavord et al., 1997). Twenty four hour air samples were collected between October 2007 and January 2011 from living rooms of properties, 0.5-1.0 m from the floor. Air samples were collected using a continuous recording air sampler and multi-vial cyclone sampler (Burkard Manufacturing Co., Rickmansworth, UK) at 10 L/min and 16.5 L/min for standard microscopy and MSQPCR analysis respectively. Dust samples were collected immediately following completion of air samples by 5 min vacuum of the living room floor, conducted as previously described (Vesper et al., 2005) using a Victor V9 industrial tub vacuum, containing a Mitest sampler and filter insert (Indoor Biotechnologies, Warminster, UK) inside the wand of the vacuum. Air and dust samples were sealed in airtight containers and stored at -20°C prior to use. Fine dust was collected using sterile, 100 mm, 255 µm aperture sieves (Fisher Scientific UK Ltd., Loughborough, UK), with gross and net weight of dust recorded and final aliquots of 5 mg (± 0.5 mg) fine dust weighed into 2 ml screw cap tubes containing 600 mg (± 60 mg) 212-300 µm sterile acid-washed glass beads (Sigma-Aldrich Company Ltd, UK) and stored at -20°C.

### Sputum culture

Isolation of filamentous fungi from sputum was performed as described previously (Pashley et al., 2011, Fairs et al., 2010a). Briefly, sputum was separated from saliva and 170 mg (± 80 mg) neat plug inoculated onto potato dextrose agar plates supplemented with 16 µg/ml chloramphenicol, 4 µg/ml gentamicin and 5 µg/ml fluconazole. Plates were sealed and incubated at 37°C for up to seven days, with frequent observations. Isolates of *A. fumigatus* were identified based on colonial and microscopic features. Other (non-*A. fumigatus*) fungi were identified to the level of genus or closely related genera, followed by DNA extraction and sequencing using primers targeting the large subunit (Issakainen et al., 1999) or internal transcribed spacer (White et al., 1990) regions of the nuclear ribosomal operon (Agbetile et

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al., 2012). DNA was extracted from pure isolates of fungi using the DNeasy plant mini kit (Qiagen, West Sussex, UK), following manufacturer's instructions, with the inclusion of an initial 2 minute bead beating step. PCR conditions were as described previously (Issakainen et al., 1999) and DNA sequences were determined using BigDye-Terminator v3.1 chemistry with 3730 sequencers (Applied Biosystems, Warrington, UK). Returned sequences were manually inspected and trimmed, followed by comparison with known sequences in GenBank (March 2010) using the BLAST<sub>N</sub> method (Altschul et al., 1990). Closest taxonomic match was reported.

#### Quantification of fungi from air and dust samples

Concentrations of airborne *Asp/Pen*-type conidia were determined through microscopic analysis at a magnification of 630x. Slides were stained with polyvinyl lactophenol cotton blue and a single longitudinal traverse of one field width was counted down the centre of each slide, with concentrations adjusted to conidia/m<sup>3</sup> air in a standard method described previously (Corden et al., 2001; BAF, 1995).

We analyzed concentrations of *A. fumigatus*, *A. niger*, *A. flavus* and *P. chrysogenum* in air and dust samples by MSQPCR as described previously (Haugland et al., 1999; Brinkman et al., 2003), using validated assays (Haugland et al., 2004). These assays are known to also amplify close relatives of *A. fumigatus*, *A. niger* and *A. flavus* (amplifying *Neosartorya fischeri*, *Aspergillus awamori* and *foetidus*, and *A. oryzae* respectively) (Haugland et al., 2004). For ease of reporting, the assays are referred to as the target fungi used in initial calibrations: *A. fumigatus* (#7097, National Collection of Pathogenic Fungi; NCPF), *A. niger* (#16888, The American Type Culture Collection; ATCC) *A. flavus* (#16883, ATCC) and *P. chrysogenum* (#2715, NCPF). Air and dust samples were processed in an identical manner



with the exception that sterile glass beads and 100 µl sterile 0.5% Tween<sub>80</sub> were added to original air sample vials and vigorously mixed prior to DNA extraction in the original tubes, whereas 5 mg fine dust was transferred into 2 ml screw top vials for extraction. *Geotrichum candidum* (#7863, University of Alberta Microfungus Collection and Herbarium; UAMH) was spiked into air and dust as an internal reference (Haugland et al., 2002, Haugland et al., 2004), followed by extraction of DNA according to the method used for fungal isolates from sputum. MSQPCRs were run in the Applied Biosystems FAST 7500 PCR instrument (Applied Biosystems, Warrington, UK).

### Data analysis

All data was analyzed using GraphPad Prism (Version 5; GraphPad Software Inc, CA, USA). Fungal spore concentrations were shown to be non-normal using the D'Agostino and Pearson omnibus normality test and also had zeros in the dataset. Raw data was log-transformed to normalize ( $\text{Log}_{10}(Y+1)$ ; where  $Y = \text{fungal conidia}/\text{m}^3 \text{ air}$ ). Parametric data was expressed as mean with standard error of the mean and analyzed using Bonferroni-corrected one way analysis of variance (ANOVA) and two-tailed, unpaired t-tests. Non-parametric data was expressed as median with interquartile range and analyzed by Dunn's-corrected Kruskal-Wallis, Mann-Whitney, Fisher's exact, Cronbach's alpha reliability coefficient and Spearman's rank correlation coefficient.

## Results

### Study cohort

Around half of patients recruited into the study were male, with a mean age of 56.8 years.

The majority of patients were on GINA step 4 or 5 treatment and prescribed high dose

inhaled steroids (Table 1). 56.4% of patients had evidence of bronchiectasis and 57.9% of patients were atopic to common aeroallergens (not including the fungal panel) and 53.4% of patients were fungal sensitized. 29 patients had evidence of IgE sensitization to *A. fumigatus* (wheal  $\geq$  3 mm and/or *A. fumigatus*-IgE  $>0.35$  kU/L), 11 patients had only evidence of IgG sensitization (*A. fumigatus*-IgG  $>40$  mg/L) and 18 patients were non-sensitized.

44.8% of patients had a positive sputum culture for *A. fumigatus*; 58.6% for any filamentous fungi (Table 1), of which 13.8% were isolated in the absence of *A. fumigatus* co-culture.

### Fungal concentrations

There was a trend for higher levels of airborne *Asp/Pen*-type conidia, as determined by microscopy, in homes of asthma patients with a positive sputum culture of *A. fumigatus* in comparison to culture negative patients although this did not quite reach significance ( $P = 0.058$ ; Figure 1a). Specifically targeting airborne levels of *A. fumigatus* by MSQPCR demonstrated significantly higher airborne levels in homes of *A. fumigatus* sputum culture positive patients ( $P = 0.040$ ; Figure 1b). No association was found between airborne levels of *A. fumigatus* and sensitization to *A. fumigatus* (Figure 1c). Airborne concentrations of *A. fumigatus* conidium equivalents detected by MSQPCR (per m<sup>3</sup> air) were positively, albeit weakly, correlated with concentrations of *Asp/Pen*-type conidia (per m<sup>3</sup> air) determined by microscopic analysis ( $r = 0.297$ ,  $P = 0.030$ ; Figure 2). This significance of the association was affected by two extreme data points and reanalysis following removal of these data points removed the significance of the association; however, the correlation remained positive ( $r = 0.219$ ). There was no association between either *A. fumigatus* isolation from sputum or sensitization and dustborne levels of *A. fumigatus* in patient homes (Figures 3a and 3b respectively). There was no correlation between air and dust concentrations of any target

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fungi examined. Concentrations of *A. fumigatus* concentrations in air and dust were negatively correlated; however, this was not significant ( $r = -0.203$ , Figure 4). Removal of two extreme datapoints favouring a positive association strengthened the negative correlation, which then became significant ( $r = -0.332$ ,  $P = 0.018$ ). Duplicate dust samples were analyzed from ten of the 58 homes, and demonstrated an acceptable level of consistency in *A. fumigatus* dustborne conidium equivalents between duplicate 1 mg samples using Cronbach's alpha reliability coefficient ( $\alpha = 0.799$ ,  $P = 0.013$ ).

In contrast to *A. fumigatus*, the number of sputum culture positive patients for other target fungi was too few to permit statistical analyses. The descriptive statistics for concentrations of fungi in air and dust samples from homes for all target fungi are presented in Table 2.

## Discussion

This is the first study to show a direct link between indoor airborne fungal spore concentrations and detection in sputum, indicating airways colonization in patients with asthma.

*Aspergillus* and *Penicillium* conidia can be found in high concentrations indoors, even in non-complaint homes without obvious damp (Ren et al., 2001; Fairs et al., 2010b) and fungal sensitization may be attributed to either environmental exposure or internal exposure to colonising fungi (Denning et al., 2006); however, it is unclear whether isolation of multiple fungi from sputum indicates environmental contamination, impaired clearance or elevated exposure. This study has shown significantly higher levels of airborne *A. fumigatus* conidium equivalents in homes of asthma patients with a positive sputum culture for the fungus. There was a trend towards higher *Asp/Pen*-type conidia, as determined by microscopy, in homes of asthma patients with a positive sputum culture for *A. fumigatus*, however, these levels did not

exceed typical ranges (Fairs et al., 2010b). No direct association was found between *A. fumigatus* concentrations in air or dust samples from the home and *A. fumigatus* sensitization, nor was a relationship found between dustborne levels of *A. fumigatus* and detection in sputum.

Whilst our method of sputum culture does not enable quantitative analysis of fungi in sputum, the method has been shown to be superior to standard methods employed in routine clinical laboratories (Pashley et al., 2012). Now that efficient DNA extraction procedures from fungi in sputum are available (Baxter et al., 2011), future studies comparing environmental exposure levels to fungi with fungal load in sputum would be of benefit. Environmental and sputum sampling were not conducted at the same time due to the logistics of organizing visits, and outdoor fungal concentration data were not included in this study; however, we have shown previously that indoor levels of *Asp/Pen*-type conidia do not exhibit seasonal variation and exhibit an independence of outdoor concentrations (Fairs et al., 2010b) and that there is substantial agreement between repeat sputum samples up to six months apart (Fairs et al., 2010a). Furthermore, *Asp/Pen*-type conidia may comprise numerous morphologically similar spores from different fungi, possibly also from genera other than *Aspergillus* and *Penicillium*, with differences in preferred growth substrates and conditions, and indoor or outdoor predominance. Therefore, incorporation of outdoor data for *Asp/Pen*-type conidia may not be helpful. Whilst species-specific analysis of concurrent indoor and outdoor concentrations of fungi by MSQPCR was not investigated in this study, the majority of fungal species (including *A. fumigatus*) have been shown not to correlate in indoor and outdoor samples, suggesting that incorporating outdoor fungal concentrations in analyses of indoor levels may generate misleading results (Meklin et al., 2007). However, *A. fumigatus* can reach very high levels outdoor, surrounding compost piles for example (Fischer, 2008,

Taha, 2006); therefore, whilst windows were kept closed during sampling, influence of outdoor concentrations on indoor levels cannot be ruled out.

It is difficult to attribute clinical relevance to home mold concentration data for people with asthma who were sputum culture positive for non-*A. fumigatus* fungi due to low detection rates in sputum. A larger study would be required to address longitudinal aspects of fungal exposure and colonization by fungi other than *A. fumigatus*. Furthermore, genotyping of environmental and clinical isolates to confirm matching strains in the home and respiratory samples would be necessary to confirm a direct link.

Many previous studies of indoor mold concentrations have focused on the development of asthma and allergy in children. A small pilot study investigating fungal concentrations in thirteen homes of adults with and without allergic bronchopulmonary aspergillosis concluded that host susceptibility was more important than environmental exposure; however, after sensitization, environmental exposure to *Aspergillus* may exacerbate symptoms, particularly over the winter months (Vernon et al., 1980). Another study showed culture of *Cladosporium* and *Alternaria* from nasal lavage to reflect environmental exposure in adults (Kostamo et al., 2005).

No associations were found between the presence of fungi in dust and either sputum culture or sensitization in this study. Dust samples are commonly utilized for investigations of environmental exposures and respiratory symptoms due to the ease of sampling and potential for a large quantity of sample material, and dustborne exposures to fungi have previously been associated with post-building occupancy onset asthma in adults (Park et al., 2008) and childhood asthma (Reponen et al., 2011; Vesper et al., 2006). More specifically, airborne

*Penicillium* and dustborne *Alternaria*, *Cladosporium* and zygomycete concentrations have been associated with lower respiratory tract illness in children within the first year of life (Stark et al., 2003), and remediation of mold and moisture sources has been shown to reduce exacerbations and symptoms of asthma in children (Kercsmar et al., 2006). In contrast, exposure to a large diversity of fungi on farms has been associated with a reduced risk of asthma in children (Ege et al., 2011).

The link between sputum culture and indoor mold concentrations shown in this study is a very interesting observation, particularly taking into consideration alternative sites of exposure, such as the work or garden environments, and potential confounding factors. Whilst data is available for the duration of asthma of patients sensitized to *Asp/Pen*-type fungi, there is no way of knowing when there might have been initial exposure, infection or airways colonization, or how long the patients have been sensitized. Alterations of properties to reduce allergen exposure, such as by removal of carpets may also have a large effect on indoor fungal concentrations.

Few studies have investigated indoor mold concentrations in the UK. *Penicillium* and *Cladosporium* species have been shown to predominate (Hunter et al., 1994) and MSQPCR comparing fungal concentrations in house dust in the USA with 11 homes in the UK showed that the majority of 81 fungal species did not differ, with the exception of 13 (predominantly *Penicillium*) species (Vesper et al., 2005). *P. chrysogenum* and *A. niger* conidium equivalents detected in our study were comparable to those previously reported (Vesper et al., 2005) in British homes (log geometric mean conidia per 5 mg dust = 1.457 versus 1.467 (reported previously) for *P. chrysogenum* and 0.466 versus 0.223 for *A. niger*); however, concentrations of *A. fumigatus* were much higher in our study in comparison to the previous

study (log geometric mean = 1.830 versus -0.0149) and *A. flavus* concentrations were lower (-0.039 versus 0.240) (Vesper et al., 2005).

The positive correlation between levels of *Asp/Pen*-type spore concentrations analyzed by microscopy and *A. fumigatus* conidium equivalents analyzed by MSQPCR is encouraging; however, some data did not correlate, and the significance of the association was affected by two extreme data points favouring the correlation. High *Asp/Pen*-type concentrations predominated by species not measured in this study, suboptimal DNA extraction or inhibition of amplification could explain high *Asp/Pen*-type and low *A. fumigatus* concentrations; vice versa contradictions in the data may be explained by differences in orifice size and presentation for the two samplers, affecting efficiency of collection (Khattab et al., 2008).

No significant correlations were found between concentrations of *A. fumigatus* or the other three target fungi in air and dust samples. It is generally accepted that air sampling gives a measure of current exposure, whereas dust samples can reflect long-term exposure (Portnoy et al., 2004) and many studies focus on dust sampling for environmental monitoring. This study has shown that dust sampling alone could miss potentially important observations, and that ideally both air and dust samples should be analyzed together. Fungal concentrations determined by dust sampling will be affected by changes in flooring, humidity levels and differing levels of cleaning activities within properties. Furthermore, it is difficult to ascertain actual exposure from dust sources of fungi. Similarly, airborne levels of fungi will be greatly affected by numerous factors including activity levels in a property, ventilation rates, presence of potted plants, and differences in lifestyle contributing to elevated humidity. Whilst not shown in this study, elevated concentrations of fungi in dust indicating long-term exposure may give better insights into the development of sensitization; whereas airborne

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concentrations may better reflect what is actively inhaled and provide insights into airways colonization. It is unclear whether airways colonization transpires through long-term exposure to moderate levels of fungi, through a single exposure to very high concentrations, or a combination of the two. Longitudinal studies air and dust sampling in combination with, measures of airways colonization and fungal sensitization would be required to investigate this further. No guidelines are currently available for acceptable levels of fungal contamination of properties and this study provides a small step towards identifying levels which may pose elevated risk to health. In conclusion, we have demonstrated a direct link between elevated airborne concentrations of *A. fumigatus* in the home environment and a positive sputum culture for the fungus indicating airways colonization. Future studies will be required in order to confirm this finding, and genotyping studies should be undertaken to demonstrate the genetic relationships between fungi present within the home environment and those found in respiratory samples.

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**Table 1** Study cohort demographic data and clinical characteristics

Characteristic	Result
Gender male, n (%)	30 (51.7)
Age, mean yr (range) <sup>a</sup>	56.8 (24-84)
Smoking, pack yr <sup>a</sup>	0 (0-5.75)
Post bronchodilator FEV <sub>1</sub> <sup>a</sup>	75 (51.75 – 103.0)
Age of asthma onset, yr <sup>a</sup>	35 (3.5 – 51.5)
Duration of asthma, yr <sup>b</sup>	24.0 (2.5)
GINA treatment n, (%)	
GINA 1-3	7 (12.1)
GINA 4	28 (48.3)
GINA 5	23 (39.7)
Inhaled corticosteroid dose (µg) <sup>b</sup>	1571 (108.9)
Bronchiectasis, n (%) <sup>c</sup>	31 (56.4)
Atopic, n (%) <sup>d</sup>	33 (57.9)
Fungal sensitized, n (%) <sup>e</sup>	31 (53.4)

Total IgE (IU/ml) <sup>a</sup>	218.5 (81.2-751.5)
<i>A.fumigatus</i> IgE (kU/L) <sup>a</sup>	0.14 (0.05-11.6)
<i>A. fumigatus</i> IgG (mg/L) <sup>a</sup>	27.55 (10.5-61.8)
Sputum culture of filamentous fungi, n (%)	34 (58.6)
Sputum culture of <i>A. fumigatus</i> , n (%)	26 (44.8)

<sup>a</sup>Median with interquartile range in parentheses

<sup>b</sup>Mean with standard error in parentheses

<sup>c</sup>Of those scanned

<sup>d</sup>To common aeroallergens, with wheal  $\geq$  3mm (not including fungal panel)

<sup>e</sup>*A. fumigatus* IgE >0.35 kU/L or positive skin prick test to any fungal allergen tested

**Table 2** Target fungi detected by MSQPCR from air (conidium equivalents/m<sup>3</sup> air) and dust (conidium equivalents/mg dust), and *Asp/Pen*-type spore concentrations (per m<sup>3</sup> air) determined by microscopy in homes of patients with asthma. Also presented are the proportion of properties in which  $\geq$ 1 conidium was observed per 24 hr air sample or 5 mg dust sample (% properties) and number of patients with a positive sputum culture for each of the target fungi (or close relative).

Target fungi	<i>Aspergillus flavus</i>	<i>Aspergillus niger</i>	<i>Penicillium chrysogenum</i>	<i>Aspergillus fumigatus</i>	<i>Asp/Pen</i> -type
Patients isolated from, n (%)	2 (3.4)	5 (8.6)	2 (3.4)	26 (44.8)	34 (58.6)
Air					
Min	0	0	0	0	0
Max	2	1	16	138	2765
Mean	0	0	2	22	268
Median	0	0	2	9	71
GM (Log GM)	0.7 (-0.162)	0.7 (-0.131)	1.6 (0.192)	10.8 (1.034)	67.2 (1.827)
% properties	14.8	18.5	90.7	98.1	93.1
Dust					
Min	0	0	0	0	n/m
Max	59	16	319	636	n/m
Mean	1	2	19	42	n/m
Median	0	0	6	11	n/m

GM (Log GM)	0.6 (-0.211)	0.8 (-0.110)	6.1 (0.782)	14.3 (1.156)	n/m
% properties	10.5	71.9	96.5	96.5	n/m

Min: Minimum, Max: Maximum, GM: Geometric mean, n/m: not measured

**Fig. 1** (a) Airborne levels of *Asp/Pen*-type conidia detected by microscopy (conidia/m<sup>3</sup> air) and (b) *A. fumigatus* conidium equivalents detected by MSQPCR in homes of patients with asthma grouped according to isolation of *A. fumigatus* from sputum (conidium equivalents/m<sup>3</sup> air); and (c) airborne concentrations of *A. fumigatus* conidium equivalents in homes grouped according to *A. fumigatus* sensitization of patients (conidium equivalents/m<sup>3</sup> air;  $P > 0.05$ ). Lines represent mean and standard error of the mean.

**Fig. 2** Correlation between *Asp/Pen*-type conidia quantified by microscopy (conidia/m<sup>3</sup> air) and *A. fumigatus* conidium equivalents quantified by MSQPCR (conidium equivalents/m<sup>3</sup> air);  $n = 54$ .

**Fig. 3** Dustborne levels of *A. fumigatus* conidium equivalents (per mg dust) in homes of patients with asthma, grouped according to (a) isolation of *A. fumigatus* from sputum (lines represent median with interquartile range;  $P > 0.05$ ), and (b) *A. fumigatus* sensitization of patients (lines represent mean with standard error;  $P > 0.05$ ).

**Fig. 4** Correlation between *A. fumigatus* conidium equivalents detected per cubic metre of air and per mg dust;  $n = 53$ ,  $P > 0.05$ .







