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ALLERGENS (RK BUSH AND JA WOODFOLK, SECTION EDITORS)

Indoor Fungal Exposure and Allergic Respiratory Disease

Nicholas J Osborne[1,2] · **Christopher R Thornton**[3] · **Richard A Sharpe**[2]

Abstract A gathering body of evidence has repeatedly revealed associations between indoor fungi and initiation, promotion, and exacerbation of allergic respiratory disease. The relationship between the exposure and outcome are complicated by the difficulties in measuring both exposure and outcome, the multifactorial nature of the disease, and the wide range of potential confounders. New technologies are becoming available that may enable better measurement of exposure and tighter case definitions so as to build more confidence in the associations discovered. The growing strength of the evidence base will aid the design of future public health interventions and generate new hypotheses on the cause of the rapid increase in allergic respiratory disease prevalence.

Keywords Allergy · Fungi · Indoor fungal exposure · Asthma · Allergic respiratory disease · Environment and allergy · Epidemiology

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✉ Nicholas J Osborne
nicholas.osborne@sydney.edu.au

Christopher R Thornton
c.r.thornton@exeter.ac.uk

Richard A Sharpe
r.sharpe@exeter.ac.uk

[1] Clinical Pharmacology and Toxicology Research Group, Discipline of Pharmacology, Sydney Medical School, The University of Sydney, Sydney, NSW, Australia

[2] European Centre for Environment and Human Health, Knowledge Spa, Royal Cornwall Hospital, University of Exeter Medical School, Truro, Cornwall TR1 3HD, UK

[3] Biosciences, College of Life and Environmental Sciences, University of Exeter, Stocker Road, Exeter EX4 4QD, UK

Introduction

Evidence to Date

The ability for the human immune system to become sensitized to fungi and to have this progress to an allergic reaction (predominantly type 1 hypersensitivity) has been known for a number of decades [1, 2]. This source of allergen exposure is thought to be seriously underestimated, and has remained unacknowledged until more recently, both in its prevalence and economic effect [3]. A series of epidemiological studies examining the role of housing and the indoor environment have revealed the importance of dry environments to limit the risk of asthma and other immune system-related diseases, with the majority of adverse health effects kept at bay by maintaining a relative humidity indoors between 40–60 % [4]. The prevalence of this disease group was driven by a number of factors and careful dissection of the collected data has led to an increasing evidence base suggesting linkages between indoor dampness, fungi, and human health.

Allergic Respiratory Disease

Allergic respiratory disease is a group of allergic diseases with similar pathological pathways and symptoms and include the highly prevalent hay fever (or pollinosis to include non-grass pollens), allergy to aeroallergens that enter the respiratory tract (including allergic rhinitis), and asthma. Worldwide, there has been an increase in atopic diseases such as hay fever and asthma over the last 20 years, although asthma prevalence, but not allergy, appears to have plateaued [5]. Symptoms range from red eyes, itchiness, and runny nose for the majority of routine hay fever/allergy sufferers, through to eczema, hives, or asthma attacks with increasing severity of allergic reactions and associated socio-economic impact on health

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services. It should be noted that these symptoms are indistinguishable from exposure to other allergens such as pollen, house dust mite, animal dander, or insect allergens. The majority of interactions between fungi and allergy are thought to be via the IgE/Th2 pathway. In rarer instances, exposure to indoor fungi may also be responsible for increased infection and exposure to toxins, and/or harmful metabolic by-products, but a strong evidence base is lacking [6, 7]. These may include chemical moieties such as ketones, aldehydes, and alcohols [8]. While exposure to a diverse array of microorganisms is important in the regulation of the immune system, the timing and extent of exposure appears to be critical in the risk of developing allergic diseases. A high proportion of asthma sufferers (66 %) have been reported as being sensitized to one or more fungi, as measured by skin prick tests or serum IgE analysis [9].

It should be noted that this group of diseases are defined as complex diseases with multiple genetic and environmental risk factors that also overlap into gene:environment interactions such that there will never be a single "cause" of allergy or asthma. This complex picture is further complicated by similar and multiple risk factors and confounders. For instance, the presence of a damp environment in which fungi can thrive is also the environment beneficial for the reproduction of house dust mite (a common source of allergen for asthma sufferers), growth of bacteria, and the production of volatile organic compounds. Outside pollens and fungal spores have been found to vary across Europe [10] [11], and it can be presumed that there will also be geographic variation of indoor fungi. It could be hypothesized that this variation will be less than outdoor fungi, due to the narrower range of environments found in domestic dwellings, with similarities in building materials, degree of humidity, and range of temperatures [12–14].

Measurement of Allergic Disease

The complexity and heterogeneity of allergic diseases makes it difficult to characterize, and despite decades of effort, a wide range of definitions and/or validated diagnosis are used, often complicating comparisons between studies [15]. Significant improvements in case definitions have been made with the standardization of measurement of lung function via spirometry, leading to the greater understanding of asthma as a disease. Similarly, examining asthma as several component parts as defined via a number of distinct endotypes (e.g., atopic versus non-atopic) is predicted to have a similar effect [16]. Measurement of hay fever and allergy are similarly problematic, and quite often, proxies are used. These range from a variety of case definitions/data inputs from self-report, doctor-diagnosed (combination of in vivo and in vitro tests, physical examination and clinical history), skin prick testing with specific allergen, and serum measures of total and allergen specific IgE. Better measurement of disease outcome will allow a clearer picture of the relationship between indoor fungi exposure and allergic respiratory outcomes to develop and solidify putative associations. Other novel measurements are being conducted examining biomarkers of allergic inflammation such as blood eosinophils as measures of disease severity and/or symptom control [17]. Similarly, exhaled nitric oxide is now being used in cohort studies of children as a measure of lung function and to aid defining allergic asthma cases, although some measurement problems still exist [18, 19].

Allergenic Fungi and Their Allergens

Of the estimated 5.1 million species of fungi on the planet [20], only a handful of species are recognized as human respiratory pathogens [21], despite the diversity and ubiquity of fungal propagules (aerosolized spores and hyphal fragments) in inhaled air. Typically, the majority of allergenic fungi are filamentous (hyphal) organisms rather than yeasts, but certain yeast species such as the basidiomycete *Trichosporon asahii* are well known respiratory pathogens [22]. Repeated inhalation of *Trichosporon* arthroconidia can cause summer-type hypersensitivity pneumonitis (SHP), an immunologically induced lung disease, and the most common form of hypersensitivity pneumonitis (HP) in Japan. Other fungi such as the basidiomycete yeast *Cryptococcus* may also be responsible for SHP [23], although the genus, and more specifically the species *C. neoformans*, is typically associated with cryptococcosis, a disseminated and frequently fatal infection of acquired immune deficiency syndrome (AIDS) patients.

The ascomycete mold *Aspergillus* is a well-characterized pathogen of immunocompromised humans including hematological malignancy and bone marrow transplant patients, whose damaged innate immunity prevents effective clearance of infectious spores from the lungs [24]. The lung of a neutropenic patients whose front-line effector cells (phagocytic alveolar macrophages and neutrophils) are severely impaired, provides an ideal niche for fungal colonization and infection by *Aspergillus fumigatus* [24], an ubiquitous environmental mold and contaminant of buildings. This fungus accounts for the majority of the >200,000 life-threatening lung-borne infections (known as invasive pulmonary aspergillosis (IPA)) per year [25].

In addition to IPA, it has become increasingly apparent that this fungus and related species are contributing factors to other respiratory disorders [26], particularly to acute exacerbations of severe chronic obstructive pulmonary disorder (AECOPD) [27], and which, along with other molds such as *Scedosporium* spp. [28, 29] and *Geosmithia argillacea* [30], and thermophilic black yeast *Exophiala dermatitidis* [31], are colonizers of the cystic fibrosis lung causing allergic reactions and respiratory deterioration. As

an aeroallergen, *A. fumigatus* is a significant contributor to the millions of individuals globally who develop pulmonary and nasal allergies. Colonization of the airways of adults and children by the fungus can lead to severe asthma with fungal sensitisation [32], which is estimated to affect between 3.25 and 13 million adults worldwide, and contributes to the 100,000 asthma deaths annually [33]. Allergic bronchopulmonary aspergillosis (ABPA), a hypersensitivity response of the lungs, is thought to affect more than 4 million people with asthma and cystic fibrosis worldwide [34], while approximately 12 million people are thought to be affected by allergic fungal rhinosinisitis [33]. ABPA is an allergen-induced immunological disease of the lung characterized by increased IgE and IgG serum titers, enhanced peripheral blood eosinophilia and transient pulmonary infiltrates. The *A. fumigatus* allergens Asp f 2 (a 37 kDa fibrinogen-binding antigen), f 3 (a 19 kDa peroxisomal protein), and f 6 (a 26.5 kDa Mn superoxide dismutase) together react with IgE from patients with asthma and ABPA [35], suggesting a role for these allergens in disease exacerbation.

The contribution of other fungi to the global allergic disease burden is less easy to determine, but it is clear that the propagules and antigenic components of the ascomycetes *Cladosporium*, *Alternaria*, *Penicillium*, and *Stachybotrys* spp. are common contaminants of the indoor environment, and are potent inducers of allergic respiratory diseases [36]. As a common component of the microbiota of damp buildings, *Stachybotrys* species, most notably *S. chartarum*, have been implicated in sick building syndrome, a term given to severe illnesses resulting from indoor mold exposure, and remains a controversial area with a limited evidence base.

Using sera from bronchial asthmatic patients, Chou et al. (2008) demonstrated IgE binding against 36 kDa (Cla c 9 allergen) and 20 kDa serine proteases of *Cladosporium cladosporioides* and cross-reactivity with serine protease allergens from *A. fumigatus* and *Pencillium chrysogenum* [37]. Elevated IgG and IgE antibody titers against *Stachybotrys* antigen extracts have been reported in allergic patients and residents of water-damaged houses, suggesting sensitization, and hence, exposure. Sera from 50 mold-exposed humans were tested for IgG and IgE antibodies against *Stachybotrys* hemolysin and proteinaseStachyrase-A [38]. Significantly elevated titers were present in the majority of sera indicating that hemolysin and Stachyrase-A are two major allergenic components of *S. chartarum*. Stachylysin (*Stachybotrys* hemolysin) has been detected in wallboard and dust samples, implying that the allergen may be a useful indicator for assessing human exposure to *S. chartarum* and for determining presence of the fungus in the indoor environment. Wartenberg et al. (2011) have similarly shown hemolysin to be a major secreted protein of *A. fumigatus* [39].

Exposure and Disease Risk

Research into the link between allergic diseases and fungal exposures is often inconsistent, and public health data tends to focus on only a few species [40], which limits researching into potential causal pathways and the potential for exacerbation of disease through childhood and adulthood [41••]. The majority of the evidence to date has focused on a visual assessment of fungal contamination and/or the smell of odor and the risk of asthma development [42•] and/or exacerbation [43]. Both the amount and type of fungi that a person is exposed to governs the risk of allergic respiratory disease. Measurement of fungi indoors has changed in recent years with the advent of new technologies such as DNA genotyping and immunodetection. These can run in parallel and allow scientists to go beyond microscopy for genus and species identification, which did not always clearly delineate genus or species present. While the expense of these technologies is currently prohibitive for large cohort studies, it will probably be only a short period of time before they are utilized in larger studies. Advances in use of molecular techniques and use of metagenomics [40], along with a better case definition, will further research into microbial exposures and the etiology of allergic diseases.

It has been noted by a range of authors that research in this area is notoriously difficult due to the numerous factors that contribute to variation in fungal diversity and load [44]. Factors include those from built environment, behavior of residents, as well as the fungal ecology endemic to that area [45]. The diversity of bacterial communities found in dust in a survey of 1200 US homes was found to be greater than for fungal communities. Most indoor fungi were found to originate outside the home, with fungal communities in dust varying predictably with across climatic and geographical regions. Bacterial communities, but not fungal communities, varied by who made up the domicile, varying by the male:female ratio of the home and presence of pets [46]. If outdoor fungal spores are a large component of the indoor fungal aerobiota, it could be imagined that their numbers would be higher throughout the summer and autumn months (when fungal sporulation is at its greatest) and when ventilation is greater, compared to the winter months.

Measurement of Fungi

A variety of measures of fungi have been undertaken by researchers wanting to examine the potential linkages between indoor fungi and asthma/allergies [5]. The use of analytical methods to detect fungal cell wall components, mVOCs, mycotoxins, and allergens via a variety of techniques (gas chromatography/mass spectrometry, liquid chromatography/mass spectrometry, *Limulus* amebocyte lysate test, and enzyme-linked immunosorbent assay) can reveal the nature

of fungal diversity and concentrations within house dust and/or air, and have been described by others [47].

The majority of people will come into contact with fungi by breathing in spores, and perhaps the VOCs produced by the sporulating organism and water-damaged materials. Consequently only those species that sporulate at temperatures commonly associated with the domestic environment will be present. Growth and diversity of fungi colonizing indoor surfaces will be regulated by the geographic location/season (influencing air exchange rates) [48], as well as the availability of moisture [49], organic material [50], type of material and its chemical composition [51], pH and physical properties [52], which modify the growth of different hydrophilic and xerophilic fungi [53]. Understanding the variability of aerospora on concentrations of fungal components in the home versus indoor sources of fungal propagules is limited by the lack of consistent sampling techniques (measurement of house dust and airborne fungi). For example, different sampling techniques have been found to be poorly correlated due to different types or durations of potential microbial exposures from dust versus air [54].

The prevalence of indoor dampness is thought to affect around 16 % of European homes [55], and is likely to affect more homes located in areas with milder wetter climates (e.g., those associated with river and coastal areas), and in lower-income populations. The risk of dampness-related exposures in lower-income populations may persist despite efforts by housing providers to maintain properties and to introduce measures to prevent heat loss due to the risk of condensation [56]. Energy efficiency measures to prevent energy loss may in fact increase the chance of indoor fungal growth due to an increased risk of condensation in poorly heated and/or ventilated homes.

Similar to other microorganisms, research has tended so far to focus on those species that are able to be readily cultured on standard plates and media. A range of sampling techniques including fungi that settle out of the air directly onto plates, those that grow on mycological media in covered areas and those that grow on commonly used media (e.g., agar) after manual sampling are used. These methods may introduce sampling bias due to the promotion of faster growing fungi and inability to account for non-viable spores; thus, these methods may not capture all the species that humans are exposed to in the domestic environment [57]. It is also not possible to assess exposures to fungal propagules resulting from variations in relative humidity, which regulate the aerosolization, and total inflammatory potential of ultra-fine fungal particles [57, 58].

Use of the human sense of smell has also been employed to detect presence of fungi, as fungi are not always visible to the naked eye, concealed behind ceilings, wall paper and walls, where water leaks or condensation may not ways be apparent (e.g., interstitial condensation in cavities within the building fabric). Resident self-reported exposure to fungal odor [59] or a mold/musty odor [60•] has been consistently associated with the exacerbation of asthma. It has also been associated with lower lung function among non-asthmatic adults [61•]. A recent study investigating dampness-related exposures in a large representative US population found that fungal odor was implicated in childhood-allergic asthma and non-allergic asthma in adults [60•]. The presence of odor has been found to correlate well with increased severity of fungal contamination when compared with quantitative polymerase chain reaction (PCR) [54], and shown to be accurate in longitudinal studies [62]. This is particularly apparent in homes with a lack of ventilation and/or have poor ventilation practices by residents [63]. Relying on resident self-reports of the presence of odor may introduce bias, which can be reduced by home inspections with a trained investigator and quantitative PCR.

Quantitative PCR

Use of quantitative PCR allows detection of fungi at both the genus (e.g., *Aspergillus* or Penicillium) and species level (e.g., *Stachybotrys chartarum*) and overcomes ambiguities of identification inherent in culture-based microscopy. One of the limitations of this detection method is that investigators must decide beforehand which fungi to measure because of reliance on species-specific probes. Vesper et al. have developed the environmental relative moldiness index (ERMI) [12, 64]. to overcome this limitation in an attempt to deal with the complexity associated with the wide spectrum of fungi that may be present in houses. It is designed to minimize the risks associated with sampling via growth on media or swabbing visible growth on walls and allow extrapolation of exposure measurement between studies. The index is not composed of the total fungal concentration in the dust, but is used to rank houses as to their relative fungal burden, while accounting for fungi derived from water leaks and from outdoor sources, using quantitative DNA analysis of 36 species of fungi in dust samples.

Fungal-specific quantitative PCR can be used for specific identification and quantification of viable and non-viable fungal propagules and is being increasingly used because of its low detection limit and high specificity [47]. It has been used in a variety of studies, principally pediatric epidemiological studies in the USA with asthma outcomes [65, 66•] and to assess the heterogeneity of indoor fungi across different geographic locations [13, 14, 65]. Translation of the EMRI methodology outside of the USA has been achieved in France [67] and Scotland [68] with success. The index can be used to predict the risk of respiratory illness in homes and allows stakeholders to make choices about various levels of risk [69]. This is important considering the finding that high fungal diversity is linked to dampness brought on by water damage, but low fungal diversity is linked to asthma [70].

Systematic Reviews of Evidence of Association Between Indoor Fungi and Allergenic Respiratory Outcomes

The expanding evidence base provided by epidemiological studies that examine the association between indoor fungal exposure and allergic/asthmatic disease has made it possible to conduct systematic reviews and meta-analysis. A review of meta-analyses on residential exposure to damp and/or fungal contamination shows consistent and positive associations with multiple allergic and respiratory effects in child and adult populations [71]. It is important to identify which specific or aggregate indoor microbial exposures have adverse human health effects and which may have protective effects [72].

Meta-analysis of 33 US studies on the relationship between dampness and mold in homes and respiratory health effects revealed a 35–75 % increase in risk of reporting respiratory or asthma-related health outcomes, and no effect was seen only one in ten times [43]. The lower boundary of confidence interval saw a greater than 20 % increased risk of disease in most cases.

The Pollution and the Young (PATY) project was a collaborative enterprise where researchers from several comparable cross-sectional studies pooled their original data on exposures and health status for 58,561 children. The studies were carried out during 1988–1999 in ten countries in Eastern and Western Europe, Russia, and North America [73]. Positive associations between fungal exposure and increased risk of reporting children's respiratory ill-health were found, with considerable consistency across the countries sampled.

Examination of data collected in Europe from eight birth cohorts consisting of a total of 31,742 children [74•] found that exposure to visible fungi and/or dampness during the first 2 years of life was associated with increased risk of the development of asthma. Four cohorts of children exposed at 0–2 years saw an adjusted odds ratio of 1.39 (95 % CI 1.05, 1.84) for asthma and in six cohorts with symptoms of allergic rhinitis at school age (6–8 years old when exposed) with an odds ratio of 1.12 (95 % CI 1.02, 1.23). The authors do point out that drawing causal relationships is difficult, and the variability of the microbial components in indoor air continues to be a major stumbling block. Some evidence was found that early life exposure to fungi was observed to decrease the risk of sensitization to fungal allergens in children with allergic parents but was not conclusive due to the small sample size.

Quansah et al. (2012) examined the literature from 1990 to 2012 and performed a systematic review and meta-analysis with the objective of assessing the relationship between indoor dampness and fungal problems and the risk of developing new asthma [42•]. Eleven cohort and five incident case-control studies were identified. The summary effect estimates based on the highest and lowest estimates for the relation between any exposure and onset of asthma were 1.50 (95 % CI 1.25, 1.80, random-effects model, Q statistic 38.74 (16), P=0.001) and 1.31 (95 % CI 1.09–1.58, random-effects model, Q statistic 40.08 (16), P=0.000), respectively.

Sharpe et al. (2015) posed the question of whether there were genera- or species-level differences in the relationship between fungi and asthma, as opposed to "ubiquitous fungi", for both adults and children [75]. Presence of *Penicillium*, *Aspergillus*, and *Cladosporium* prior to the development of asthma symptoms were found to be associated with increased risk of respiratory ill-health. Addition of *Alternaria* to this group saw an increased risk of exacerbation of current asthma. Others have hinted at a differential species/genus effect, including an Australian study where participants whose *Cladosporium* fungal exposure doubled, had 52 % greater odds of having reported an asthma attack in the previous year [76].

Potential Drivers of Indoor Fungal Exposure

Ventilation

A complex interaction between the built environment (architectural design, type of materials, geographic, and orientation) and a variety of occupant behaviors (cooking, drying washing indoors, presence of pets and heating, and ventilation practices) regulates indoor moisture levels and risk of fungal growth. A critical factor in the regulation of moisture is the circulation of air in domestic buildings, which is seen as key to the health of the residents. Airflow lowers concentrations of indoor pollutants (dependent on outdoor levels) and prevents the build-up of moisture in rooms, where fungi can grow and spore concentrations exceed outdoor ambient conditions. The indoor temperature (ambient and dew point), indoor, and outdoor humidity levels are important determinants for moisture levels and risk of condensation. But, most (but not all) ventilation systems require an increase in heating to maintain temperature. In many European countries building regulations stipulate that a whole dwelling air exchange rate of 0.5/h is required to avoid negative health effects [77]. It has been argued that this ventilation rate may not be sufficient to account for variations in housing and occupant behaviors. Das et al. [77] found that flats/apartments require a greater air exchange rate than other builds (0.7/h). This is likely to be compounded by other behavioral risk factors such as frequency of opening windows or knowledge about necessary techniques to avoid dampness problems. While building regulations provide a baseline ventilation rate to safeguard residents in new builds (or those receiving significant renovations) in the UK, for example, they are not applied to energy efficiency measures. Reduction of carbon footprints of housing via modification of existing stock via remediation work can be achieved by sealing of chimneys, applying double-glazing, and the addition of attic and wall insulation. The risk is that these measures may reduce the whole dwelling ventilation rate

in older housing stock receiving these energy efficiency measures, which may result in reduced ventilation and unintended related consequences on resident health. This may be reduced in newly built homes of good quality, but this is not always present in homes built under high economic pressures due to demands of availability, cost, and technical ability. Loss of permeability of structure membranes has not always been matched by changes in behavior by residents to allow for reduced ventilation.

Adequate Heating and Fuel Poverty

While ventilation is an essential element for the control of moisture, this must be accompanied by adequate heating practices throughout the entire home. This is required to prevent the migration of warm moist air to colder areas of a property, where it can condense on cooler surfaces (below dew point) and promote fungal growth (may be visible or interstitial). This is a particular risk in homes with high occupancy levels, poor ventilation and heating, which can result in a high relative humidity (>70 %) and vapor pressure that elevates moisture levels [78]. The type of heating systems and fluctuations in indoor temperature have been found to modify the diversity of indoor fungi and extent of fungal contamination/sporulation [45], which typically occurs between 18 and 32 °C [79]. A worldwide public health concern is the increased prevalence of energy poverty, where residents are unable to maintain adequate warmth due to the cost of fuel. This has consequences for both the risk of condensation (and associated chemical and biological exposures) and temperature-related morbidity and mortality. Sharpe et al. [56, 80] have shown that fuel poverty increases the risk of both visible fungal contamination and the presence of an odor, regardless of energy efficiency levels, use of ventilation, and risk perception of the potential health risks. Identifying mechanisms to help address fuel poverty and further research on appropriate behavioral change interventions can help alleviate damp and cold related problems in vulnerable populations and those residing in energy efficient homes.

New Paradigms and Future Opportunities

New technologies are opening up possible research areas for more thorough and comprehensive projects both examining the association between indoor fungi and asthma/allergic disease as well as opportunities to design interventions. As DNA-sequencing costs continue to fall and availability increases, combined with new modes of examination (e.g., DNA barcoding and shotgun sequencing) further opportunities will present themselves. The complex nature of both the large number of species and the ecology of these around the various rooms in the house make it essential that researchers are well equipped for the bioinformatics challenges of handling the large amounts and complex types of data derived from DNA sampling. Some of these issues may be resolved if certain genera or species of fungi are found to be categorically more harmful than others, or biomarkers of the presence of harmful species are elucidated. Other technologies such as immunological identification of species are also predicted to become an important tool for understanding and mitigating the effects of allergenic indoor fungi. Transfer of monoclonal antibody technologies for the detection of invasive opportunistic fungi to environmental detection will add another tool in estimating exposure to fungi [29].

Due to the variable qualities of fungal allergen extracts, diagnosis and treatment options for individuals with fungal allergies have been limited. Use of molecular cloning techniques has meant the preparation of recombinant synthetic fungal allergen derivatives that may lead to the development of vaccines for the treatment of fungal allergy [81]. Advances in molecular techniques to characterize fungal exposures need to be considered alongside the utilization of epidemiological and asset management techniques to further explore linkages between a diverse array of indoor/outdoor exposures and health outcomes. Longitudinal studies are required to account for the variability of exposures (physical, biological, and chemical agents) and the interaction with the outdoor environment over time to further our understanding into the potential divergent effects on health. This has been illustrated by a recent study by Sharpe et al. [60•] who found that while fungal odor represented a respiratory health risk in children and adults, there was suggestive evidence that increased diversity of biological contaminants reduced the risks of childhood eczema and adult asthma. The findings were limited by sample size, but they support that variations in lifestyle factors, the interaction with communities (internal and external to the family), and the natural versus built environment are important factors for allergic diseases at the population level. Larger studies and models of higher complexity are required to further our understanding of factors regulating the development and/or exacerbation of these complex heterogeneous diseases. Further research in this area should include the role of allergic versus non-allergic pathways, which involves the assessment of specific intrinsic (genes) and extrinsic (surrounding environment, birth mode, diet, or antibiotics) pathways involved in disease pathogenesis and development at the population level [60•]. A truly multidisciplinary approach involving housing/environmental/molecular and healthcare professionals may shed some light on the important factors regulating the health of the human microbiome [60•].

Conclusions

New technology is driving discovery in the relationship between indoor fungi and allergenic respiratory disease. New

techniques will aid the unraveling of the complexities associated in discovering pathological pathways from exposure to increased risk of disease.

Compliance with Ethical Standards

Conflict of Interest Drs. Osborne, Thornton, and Sharpe declare that they have no competing interests.

Human and Animal Rights and Informed Consent This article does not contain any studies with human or animal subjects performed by any of the authors.

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- •• Of major importance

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