Environmental Research 144 (2016) 32–42

Contents lists available at ScienceDirect

Environmental Research

journal homepage: www.elsevier.com/locate/envres

# Identifying risk factors for exposure to culturable allergenic moulds in energy efficient homes by using highly specific monoclonal antibodies

Richard A. Sharpe [a], Kate Le Cocq [b], Vasilis Nikolaou [c], Nicholas J. Osborne [a,d], Christopher R. Thornton [e,*]

[a] European Centre for Environment and Human Health, University of Exeter Medical School, Truro TR1 3HD, United Kingdom
[b] Rothamsted Research, North Wyke, Okehampton EX20 2SB, United Kingdom
[c] University of Exeter Medical School, The Veysey Building, Salmon Pool Lane, Exeter EX2 4SG, United Kingdom
[d] Clinical Pharmacology and Toxicology Research Group, Discipline of Pharmacology, Sydney Medical School, The University of Sydney, NSW, Australia
[e] Biosciences, College of Life and Environmental Sciences, University of Exeter, Exeter EX4 4QD, United Kingdom

ARTICLE INFO

*Article history:*
Received 15 September 2015
Received in revised form
19 October 2015
Accepted 27 October 2015
Available online 5 November 2015

*Keywords:*
Allergenic fungi
Asthma
Monoclonal antibody
Antigen
ELISA

ABSTRACT

The aim of this study was to determine the accuracy of monoclonal antibodies (mAbs) in identifying culturable allergenic fungi present in visible mould growth in energy efficient homes, and to identify risk factors for exposure to these known allergenic fungi. Swabs were taken from fungal contaminated surfaces and culturable yeasts and moulds isolated by using mycological culture. Soluble antigens from cultures were tested by ELISA using mAbs specific to the culturable allergenic fungi *Aspergillus* and *Penicillium* spp., *Ulocladium*, *Alternaria*, and *Epicoccum* spp., *Cladosporium* spp., *Fusarium* spp., and *Trichoderma* spp. Diagnostic accuracies of the ELISA tests were determined by sequencing of the internally transcribed spacer 1 (ITS1)-5.8S-ITS2-encoding regions of recovered fungi following ELISA. There was 100% concordance between the two methods, with ELISAs providing genus-level identity and ITS sequencing providing species-level identities (210 out of 210 tested). Species of *Aspergillus/Penicillium, Cladosporium, Ulocladium/Alternaria/Epicoccum, Fusarium* and *Trichoderma* were detected in 82% of the samples. The presence of condensation was associated with an increased risk of surfaces being contaminated by *Aspergillus/Penicillium* spp. and *Cladosporium* spp., whereas moisture within the building fabric (water ingress/rising damp) was only associated with increased risk of *Aspergillus/Penicillium* spp. Property type and energy efficiency levels were found to moderate the risk of indoor surfaces becoming contaminated with *Aspergillus/Penicillium* and *Cladosporium* which in turn was modified by the presence of condensation, water ingress and rising damp, consistent with previous literature.



## 1. Introduction

Increased exposure to indoor damp and associated fungal contamination is a worldwide public health concern because of its association with an increased risk of allergic diseases (Fisk et al., 2007; Mendell, 2014; Quansah et al., 2012), now present in around a third of the European population (Annesi-Maesano and Moreau, 2009). Fungal growth on surfaces in homes increases resident's exposures to elevated concentrations of spores and hyphal fragments (Sharpe et al., 2014c), which in turn is influenced by the type of material (Andersen et al., 2011), moisture (Flannigan et al., 2011), indoor air velocity, and the types of fungi present (Mensah-Attipoe et al., 2014). There is limited research assessing how the interaction between occupant behaviours and the built environment regulates the diversity of allergenic fungi (Sharpe et al., 2014b). This is important to consider because different genera of allergenic fungi are associated with the development (Reponen et al., 2011) and exacerbation of asthma (Sharpe et al., 2014b), and a phenotype of severe asthma in sensitised individuals (Denning et al., 2014; Denning et al., 2006). Despite current knowledge of the involvement of fungal allergens in the pathophysiology of allergic diseases, fungi as a prominent source of allergens are still largely neglected (Crameri et al., 2013).

Culturability of fungal propagules has a profound effect on the production of allergens, with culturable spores having a greater potential to evoke inflammatory disease than dead ones when deposited in the respiratory tract (Lee et al., 2006; Sercombe et al., 2004). Furthermore, increased allergen production during spore germination has been demonstrated (Green et al., 2003; Lee et al., 2006; Mitakakis et al., 2001; Sercombe et al., 2004). Consequently,

*Abbreviations:* ELISA, Enzyme-Linked Immunosorbent Assay; IAQ, Indoor air quality; mAb, Monoclonal antibody; OR, Odds ratio; SAP, Standard assessment procedure; VOC, Volatile organic compound

* Corresponding author.
*E-mail address:* c.r.thornton@exeter.ac.uk (C.R. Thornton).

http://dx.doi.org/10.1016/j.envres.2015.10.029
0013-9351/

methods of identification are needed that extend beyond categorisation of fungal contamination by the presence of dampness and visible fungal growth, to detection of culturable moulds known to cause allergic reactions such as *Aspergillus* (Gravesen et al., 1999; Patterson and Strek, 2010; Shen et al., 2007), *Penicillium* (Gravesen et al., 1999; Shen et al., 2007), *Ulocladium* (Gravesen et al., 1999; Kaur et al., 2010), *Alternaria* (Breitenbach and Simon-Nobbe, 2002), *Epicoccum* (Bisht et al., 2000), *Cladosporium* (Breitenbach and Simon-Nobbe, 2002; Gravesen et al., 1999), *Trichoderma* (Lübeck et al., 2000), and *Fusarium* species (Verma and Gangal, 1994). Identifying risk factors that promote the growth of these allergenic fungi can inform housing interventions aimed at ameliorating disease symptoms in susceptible populations. Tailored housing improvements offer a cost-effective approach to delivering healthcare to individuals suffering from moderate to severe asthma (Edwards et al., 2011) and improving lung function of individuals residing in, for example, mould contaminated water-damaged homes (Norbäck et al., 2011).

The Environmental Relative Moldiness Index (ERMI), which encompasses a range of fungal indicator species (Vesper et al., 2007) has been adopted, albeit principally in the US, as a method for categorising the extent of indoor fungal contamination. The index has been used to determine levels of risk to fungal exposure in the home and to predict the occurrence of illness in homes (Vesper et al., 2006). Based on mould-specific quantitative PCR (MSQPCR), it determines loads of fungal DNA in dust samples and is being increasingly used because of its low detection limit and high specificity (Méheust et al., 2013). While MSQPCR is precise, it is based on nucleic acid-based detection methods that are unable to differentiate between DNA derived from live and dead propagules. Furthermore, the US Environment Protection Agency has not validated or peer reviewed MSQPCR or ERMI for public use, considering it to be a research tool only, despite firms offering remediation services based on results of ERMI surveys.

No studies have investigated the combined use of culture and well-characterised fungal-specific monoclonal antibodies (mAbs) as a means of detecting and identifying culturable allergenic fungi indoors, or to use this approach to determine potential risk factors that regulate their occurrence in homes. In this study, we combine asset management, epidemiology, detection using mAb-based ELISA and validation using Internal Transcribed Spacer (ITS) sequencing of fungi, to determine potential risk factors that promote the growth of culturable allergenic *Aspergillus*, *Penicillium*, *Ulocladium*, *Alternaria*, *Epicoccum*, *Cladosporium*, *Trichoderma*, and *Fusarium* spp. in energy efficient homes. This is the first time, to our knowledge, that mAbs have been used to assess how demographic and environmental factors modify the growth of these allergenic moulds.

## 2. Methodology

### 2.1. Study population

Ethical approval for this cross sectional study was granted by the University of Exeter Medical School, application number 13/02/013. The Cornish Health project was conducted during 2013 and 2014 in collaboration with a social housing association located in the SW of Cornwall, England which manages around 4,000 social housing properties (Sharpe et al., 2015b). We worked closely with the social housing associations customer services contact centre to recruit study participants from the target population (customers of the social housing association) (Sharpe et al., 2015b). Using a standard template (Appendix A), customers from 83 social housing properties (those who contacted customer services between April and September 2013) were randomly selected and asked whether they wished to participate in the Cornish Health project. Interested participants were subsequently sent a covering letter and information sheets, and were then contacted by telephone five days after the postage date of each letter to arrange a home visit. Written consent was obtained using a form containing a series of scripted questions concerning participant involvement in various elements of the study. We used face-to-face questionnaires to collect demographic, behavioural and health data from participating adults (Appendix B), which was followed by an environmental survey using a standardised template (Appendix C).

### 2.2. Property data

Property records from the social housing association were obtained from the asset management and stock condition database in February 2014 and merged using a unique household identifier. Data included residency period, property age and build type, type of heating, glazing, insulation levels, energy efficiency ratings and date of any property upgrades. Energy efficiency ratings were calculated according to the Government's Standard Assessment Procedure (SAP). SAP 2009 was used for compliance with building regulations in England & Wales (BRE, 2013) for new builds (Part L1A) and existing buildings (Part L1B). It is the chosen methodology for delivering the EU performance of building directive (EPBD) and is used in the calculation and creation of Energy Performance Certificates (Kelly et al., 2012). SAP is calculated for both new and existing builds, and ranges from 0 to 120 with 120 representing the highest energy efficiency rating. SAP ratings were provided by the social housing provider and were auto-assessed using RDsap 9.91 (BRE, 2014) and taken from new build energy assessments (Department of Energy & Climate Change, 2014).

### 2.3. Socio-economic status (SES)

We obtained the IMD scores for 32,482 LSOAs (Large Super Output Areas) in England and Wales: each area contains a mean population of between 1000 and 1500 people (ONS, 2014). The score uses the English Indices of Deprivation 2010 to identify areas of England experiencing multiple aspects of deprivation, and were merged with our data using property full postcodes.

### 2.4. Questionnaire data

Questionnaires were designed to collect data on participant demographics on all occupants and environmental exposures thought to influence the risk of asthma initiation and/or exacerbation (Dales et al., 2008; Gaffin and Phipatanakul, 2009). Boxes were provided for either partner in the household to provide answers (Appendix B). Questions covered participant age, sex, height, weight; smoking status; employment; cleaning regimes; number of rooms carpeted; pets; health data on asthma, allergy and chronic bronchitis or emphysema; heating/ventilation regimes and whether participants thought damp/mould impacted their family's health. We modified the LARES project questionnaire (Ormandy, 2009) and ISAACs definitions (Asher et al., 1995) to assess the exacerbation of wheeze, and then current asthma by asking participants if they had seen a doctor in the last 12 months and/or take medication for asthma.

### 2.5. Environmental data

Home surveys were conducted throughout the year with 10, 5, 3, 10, 2, 2, 2 and 7 visits being carried out during April, May, June, July, May, September, December and January 2013/14, respectively. A trained investigator (RS) carried out environmental surveys using a Protimeter MMS2 damp meter Model: BLD8800 (General