

ENVIRONMENTAL DETERMINANTS

Associations between Quantitative Measures of Fungi in Home Floor Dust and Lung Function among Older Adults with Chronic Respiratory Disease: A Pilot Study

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Objective. Many fungi may cause allergic reactions and increase asthma symptoms prevalence and severity. One susceptible, vulnerable population subgroup of increasing size in industrialized countries and of public health concern who spends the majority of their time at home is older adults. Older adults diagnosed with chronic obstructive pulmonary disease (COPD) are at risk of exposure to fungi. Currently, species identification is based on observation of microscopic and macroscopic morphologies, which may underestimate concentrations compared to quantitative polymerase chain reaction (qPCR)-based measurements which are toxicologically more relevant to exposure science. **Methods.** This article analyzes quantitative indoor exposure data on fungi in floor dust (cells/cm² floor) by real-time qPCR-based detection with quantitative outcome data via field spirometry in a pilot community-based study in Visalia, Tulare County, California, between July 2009 and January 2010. Subjects ($n = 9$, five females, four males) were Caucasian, English-speaking, nonsmoking older adults with doctor-diagnosed asthma and/or COPD. **Results.** While certain results did not reach statistical significance ($p \leq .10$) due to sample size—overall and by gender—we found consistent trends and statistically significant associations for total fungal DNA (summer data) with forced vital capacity and forced expiratory volume in 1 second. **Conclusions.** The results of this pilot study are novel and suggest adverse effects of exposure inside homes to certain fungal species. This pilot study supports the need for larger prospective epidemiologic studies of older adults with asthma and/or chronic bronchitis based on quantitative environmental and clinical measures.

Keywords asthma, COPD, house dust, mold, qPCR, wipe sampling

INTRODUCTION

Fungi (mold) on surfaces and as airborne spores are ubiquitous in nature and common inside and outside residential microenvironments across seasons (1). Chronic fungal exposures are important in understanding the epidemiology of respiratory diseases (2). Other cofactors indoors, including dampness and the presence of carpets, have been associated with higher airborne fungal spore counts in living rooms, family rooms, kitchens, bathrooms, and bedrooms (3). Damp homes, especially with visible mold and/or mold odor, have repeatedly been shown to be associated with respiratory symptoms of bronchitis and asthma (2, 4–12). Thus, dose responsiveness of exposure to dampness is likely mediated by fungal spores (13). To date, in studies involving adults with asthma, exposure to fungi has been typically assessed through settled dust samples (2, 3, 7, 14–17) as opposed to short-term air samples, of viable and/

or nonviable spores, given the higher fungal analysis detection limits (DL), the desire to estimate exposure over a time period compared to a short duration, and in consideration of required resources available.

To investigate which fungi are specifically associated with adverse health outcomes, one must be able to identify and quantify individual fungal genera and species. Conventional techniques for identifying fungi rely on the ability to culture fungi in the laboratory on plates after conducting active air or settled dust sampling on specific sampling media, and this may underestimate the concentration and diversity of fungal spores that are capable of eliciting health effects (18). Alternatively, the quantitative polymerase chain reaction (qPCR) method of detection is sensitive, rapid and can be applied to situations where low counts of sampled fungi are available (19). As fungal identification is based on specific DNA sequence, qPCR can provide precise identification of fungal agents (20). For instance, previous research successfully identified 82 different species of fungi using qPCR (20, 21).

Associations between specific fungi and human diseases are the subject of an emerging body of literature, some of which are discussed below. For instance,

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Fairs et al. (22) reported *Aspergillus fumigatus* detected in sputum is associated with neutrophilic airway inflammation and suggested airway colonization with *A. fumigatus* caused airflow obstruction resulting in asthma. Sharma et al. (23) reported a statistically significant ($p < .001$) correlation between severity of allergic disease and indoor fungi and dust mite concentrations, but not indoor pollen concentration, in homes of 90 patients in Assam, India. Specifically, they observed genus *Aspergillus* and *Cladosporium* as the dominant fungal species in these homes. A study of long-term health effects of ambient air pollution in German school children aged 5–14 years found *Cladosporium* and *Aspergillus* in home dust were associated with allergic sensitization (24). Another study of children reported *Penicillium* exposure was a risk factor for asthma and *Aspergillus* was a risk factor for atopy (25). Studies to date, overall, have focused on school-age children or families, not older adults. In addition, studies have characterized the lung health risk resulting from exposure to fungi; the risk was greater for patients with chronic obstructive pulmonary disease (COPD) (26, 27).

Therefore, we focused our pilot study on fungi measured in the residences of consenting older adults aged 55 and over with physician-diagnosed respiratory ailments, asthma and/or COPD. Using a novel modification of an existing validated wipe sampling method for heavy metals in house dust during both cooling and heating seasons, we attempted to identify and quantify estimated indoor residential exposure to fungal species with qPCR (28). In general, for this targeted pilot study population of older adults, the majority of time on weekdays is spent indoors in homes. In this article on our pilot study, we explored potential associations between fungi and selected lung function measurements, forced vital capacity (FVC) and forced expiratory volume in 1 second (FEV1), as indicators of respiratory health. Other lung function measurements examined included FEV1%. While small sample size may preclude generalized conclusions, our pilot study is novel in its focus and provides data to inform designs of rigorous, larger prospective cohort and cross-sectional studies; enhances understanding of how exposure to specific fungi affects lung function; and informs future older adult asthma education and potential interventions in residential environments to benefit public health.

METHODS

Consent forms, field data sheets, participant questionnaires, and technician walk-through surveys were described in detail elsewhere (29) and approved by the Institutional Review Board of the University of Medicine and Dentistry of New Jersey.

Sample Population, Field Sampling, and Laboratory Analysis for Fungi in Floor Dust

Fungi were measured and identified from floor dust samples conducted as a part of a community-based participatory research pilot project conducted in Visalia, Tulare

County, California, between July 2009 (cooling season) and January 2010 (heating season) (28–30). These homes represented about one-quarter of participants meeting specific selection criteria—including confirmation of having physician-diagnosed respiratory diseases, that is, asthma, bronchitis, and/or COPD. Participants provided informed consent and completed multiple baseline and respiratory health surveys conducted April–May 2009 (29). A modification to an existing wipe sampling method developed by the American Society for Testing and Materials, Method E 1728-03 (31), was used to collect floor dust. Nine homes were sampled in the cooling season, of which six homes were available for repeated measurements in the heating season. We summarized key sampling and analysis details below. For complete descriptions of the recruitment, attributes of and participation by consenting older adults with chronic respiratory disease, as well as both the field and laboratory methods, please refer to Shendell et al. (30) and Yamamoto et al. (28). We confirmed participants were nonsmokers—but some were past smokers—and not sick with a cold or virus during the seasonal sampling time periods.

Samples were taken in kitchens on relatively smooth and nonporous hard floors, that is, sheet vinyl, including those with a tiled appearance. Within individual homes, we also identified and sampled other locations with similar flooring when possible, for example, den/living room and/or family room. In five homes considered during the cooling and heating seasons, two or more sampling locations were selected. Sampled wipes were stored in screw top glass containers (USEPA class 1/2, purchased from Cole-Parmer, Vernon Hills, IL, USA) in a -20°C freezer until subsequent analysis. For this pilot study, we neither requested nor implemented a specific cleaning procedure at participant homes prior to sampling. We observed a range of personal hygiene via home upkeep among participants through the use of a technician walk-through survey; for results and details, please refer to Shendell et al. (30).

The sample wipes were analyzed for allergenic fungi by qPCR-based methods. Briefly, the TaqMan method was used for species-specific, genera-specific, and universal fungal detection and employed Afumi, Aaltr, Cclad2, Enigr, PenGrp3, and PenAsp1mgb assays (Table 1) reported by USEPA (32). The SYBR Green method was used for universal fungal detection with primers FF2 and FR1 reported by Zhou et al. (33). For PenAsp1mgb and FF2/FR1, standard curves were generated using the *A. fumigatus* DNA standard; other standard curves were produced by pure cultures of the target species. To test for PCR inhibition, seven randomly selected extracts from wipe samples were added to subsets of diluted *Epilcoccum nigrum* DNA, and standard curves were produced, but no significant inhibition was observed.

Spirometry

We conducted simple field spirometry to assess lung function with a Puritan-Bennett “Renaissance II” spirometry system and SDI Diagnostics “FloSense II” disposable mouthpieces. Specifically, for this article on our pilot

TABLE 1.—Selectivity of the qPCR assays in this study.

Assay name	Type	Fungal species
FF2/FR1	Universal	All
Aaltr	Species specific	<i>Alternaria alternata</i>
Afumi	Species specific	<i>Aspergillus fumigatus</i> , <i>Neosartorya fischeri</i>
Cclad2	Species specific	<i>Cladosporium cladosporioides</i> , svar.2
Enigr	Species specific	<i>Epicoccum nigrum</i>
PenGrp3	Group specific	<i>Penicillium chrysogenum</i> / <i>griseofulvum</i> / <i>glandicola</i> / <i>coprophilum</i> / <i>expansum</i> and <i>Eupenicillium</i> spp.
PenAsp1mgb	Universal	Universal <i>Penicillium</i> , <i>Aspergillus</i> , and <i>Paecilomyces varioti</i>

study, we assessed two lung function measures—in liters (L)—related to severity of older adult respiratory disease, FEV1 and FVC. This technique provides an objective measurement of obstruction of airways symptomatic of respiratory conditions like asthma and COPD frequently encountered in old age (34). FEV1, the maximal volume of air exhaled in the first second of forced expiration after full inspiration (35), assesses bronchial (larger airway) obstruction and hence can be used to characterize asthma as a reversible airflow obstruction (36). Note FEV1, if expressed as a percentage (%) of expected value (for age, gender, height, weight, etc. that we recorded), is also an indicator of airway obstruction. Thus, we reviewed FEV1% data. For example, in normal people, FEV1 is 70–80% of FVC; the remaining 20–30% of FVC takes a further 2–3 seconds to be expelled. Lung function measures, that is, the best of three valid trials recorded for each study participant (3–5 trials attempted per person) and conducted inside at participant homes, were used in analyses both by season and as averaged across seasons (cooling and heating) given known limitations of sample size in pilot studies. This pilot study, per IRB approval, did not specifically examine their medication use or other clinical management parameters.

Data Management and Analysis

Duplicate measurements of fungi were averaged. In study homes with multiple locations sampled beyond the kitchen floor as noted above, those two or three measurements were averaged prior to subsequent analysis. Cell counts were normalized using calculated analytic DL; details are described in Yamamoto et al (28).

We examined potential associations of fungal concentration—represented as total fungi or individual species—with FEV1 and FVC, overall and stratified by gender. Initial analyses were through inspection of graphical representations of potential associations in Microsoft Excel. Fungi cell counts were log transformed to make the distribution nearly normal; originally data were skewed to the right, in part due to higher fungi measures in one home. PenAsp1mgb and all fungi (FF2/FR1) cell counts also became nearly normal distribution after log transformation. Further statistical analysis, including generalized linear regression (PROC GEE), was then conducted using SAS v.9.2 (SAS, Cary, NC, USA).

RESULTS

Table 2 summarizes specific attributes of participants and selected lung function measures from field spirometry. Of nine participants completing comprehensive first home visits in the summer cooling season (July 2009), four (44%) were males and five (56%) were females. Their average age was 66.5 years (males = 66.2 years, females = 66.8 years). Eight respondents were white; one reported being white and of other ethnicity (mixed). Six (67%) respondents had doctor-diagnosed asthma while eight (89%) had a history of bronchitis as diagnosed by a nurse or doctor. Six (50% each gender) were able to and eligible to participate in the winter heating season (January 2010) for repeated measurements. For further details, please refer below as well as to Shendell et al. (29).

In the cooling season, Figure 1a shows the observed higher fungi counts corresponding to PenAsp1mgb and FF2/FR1 analysis for respondent homes 1, 4, and 6 compared to other homes. However, we did not observe substantial variety in fungal flora overall, except for respondent homes 1 and 6. In the heating season, Figure 1b suggested increased levels of Cclad, Enigr, and PenAsp1mgb in respondent homes 5–8 compared to the cooling season. No fungi data were available for respondent homes 1, 4, and 9 in the heating season due to loss to follow-up; reasons alphabetically were deceased, moved, or traveled out of town (29).

Subsequently, fungi corresponding to Cclad, Enigr, PenAsp1mgb, and FF2/FR1 qPCR assays were focused upon, since they were present above DL in every study home; for fungi corresponding to Afumi, Aaltr, and PenGrp3 assays, except for respondent home 6, values were <DL. Visually comparing Figures 1a–b and Figure 2, higher measured counts of Cclad, Enigr,

TABLE 2.—Summary of selected study participant lung function parameters from field spirometry in different seasons, Visalia, Tulare County, California.

Season	Participant	Gender	Age	FVC (L air)	FEV1 (L air)	FEV1 (%)
Cooling	6	Male	51	1.160	0.510	44.0
	8		63	2.020	0.740	37.0
	1		67	1.725	1.496	86.8
	2		84	1.516	0.578	37.9
	7		56	3.160	2.620	83.0
	9	Female	62	2.850	2.130	75.0
	4		64	1.590	0.670	42.0
	3		76	2.010	1.430	71.0
	5		76	1.870	1.430	77.0
	6		51	1.200	0.460	38.0
Heating	8	Male	63	1.590	0.410	26.0
	1		67	n/a	n/a	n/a
	2		84	1.610	0.500	31.0
	7		56	3.270	2.63	81.0
	9		62	n/a	n/a	n/a
	4	Female	64	n/a	n/a	n/a
	3		76	2.030	1.540	76.0
	5		76	1.960	1.430	73.0

Notes: Participants 1, 4, and 9 were only sampled in cooling season (n/a in heating season). FEV1, forced expiratory volume in 1 second; FVC, forced vital capacity; n/a, not applicable.

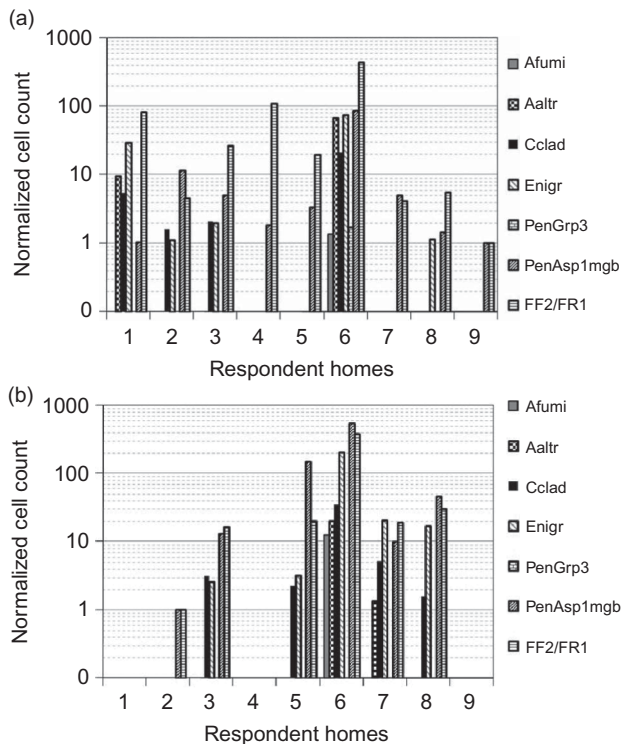


FIGURE 1.—(a) Fungi measured in house dust—kitchen and, as available, 1–2 other areas with hard, smooth sheet vinyl flooring—in participant homes of older adults with chronic respiratory disease in the summer cooling season (July 2009) in Visalia, California. Cell counts for each type of fungi were normalized by the individual DL to permit easier comparisons. Cell counts are presented on a log scale before data were log transformed. (b) Fungi measured in house dust—kitchen and, as available, 1–2 other areas with hard, smooth sheet vinyl flooring—in participant homes of older adults with chronic respiratory disease in the winter heating and rainy season (January 2010) in Visalia, California. Cell counts for each type of fungi were normalized by the individual DL to permit easier comparisons. Cell counts are presented on a log scale before data were log transformed.

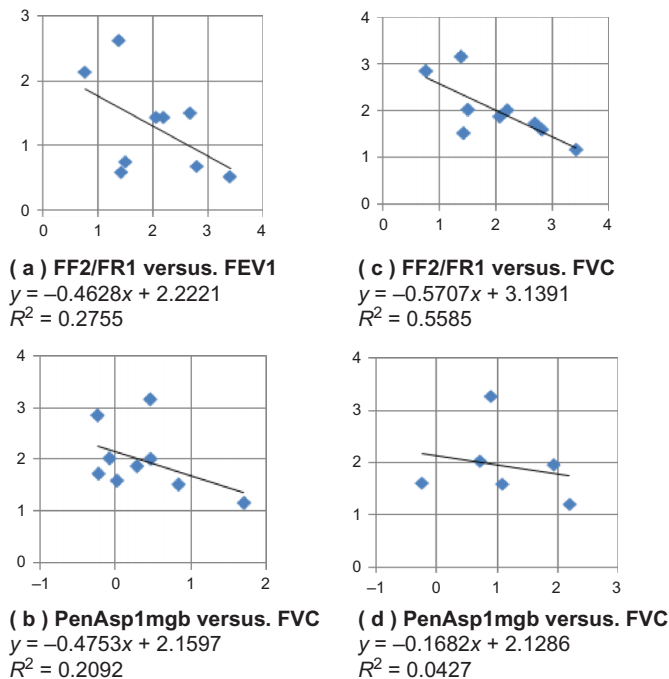


FIGURE 2.—Plots of selected fungi presented as log-transformed normalized cell count data (x-axis) and selected lung function parameters (y-axis, in liters of air), with a regression line, during the cooling season (a–c, $n = 9$ participants) and during the heating season (d, $n = 6$).

PenAsp1mgb, and FF2/FR1 in study homes appeared to correspond to lower values of FEV1 and FVC, especially for study respondent home 6 (lowest FEV1 and FVC). However, most statistical analyses showed potential associations between targeted fungi and selected lung function measures—as well as other available measures examined like FEV1%—were not substantial and/or not statistically significant after excluding respondent home 6, including after data were stratified by gender. Nevertheless, it was notable a trend of decreasing lung function measures with higher fungi counts was observed in this pilot study. Most observations were not statistically significant in part due to final sample sizes in this pilot study.

Therefore, in more rigorous statistical analyses, we plotted selected fungi against FVC and FEV1, to identify key trends for further statistical analysis, with log-transformed fungi data to best represent microbial distributions. In our study, we found log-transformed data were more approximately normally distributed, and thus focused on log-transformed data in the final analyses across seasons. We highlighted illustrative examples in Figure 2 in the cooling season (Figures 2a–c) and in the heating season (Figure 2d) with FF2/FR1 and PenAsp1mgb. Overall, though R^2 values (by PROC GEE) were low, the observed trend was decreased FVC and FEV1 with higher fungi counts.

The summary of these analyses of the fungi data is presented in Table 3, which details results from generalized linear regression models (PROC GEE) examining selected lung function measures and selected measures of fungi in indoor floor surface dust among study participants in different seasons. Statistically significant associations were observed between FF2/FR1 and FVC in the cooling season ($p = .10$) and FF2/FR1 and FEV1 in the heating season ($p = .06$) with stratification by gender. In the heating season after stratification by gender, a statistically

significant association was found with fungi data for the sum of four targeted allergenic species (Aaltr, Cclad, Enigr, and PenAsp1mgb, collectively denoted as CCASUMFUNGI) with FEV1 ($p = .05$). Overall, the trend was for decreasing FVC and FEV1 with higher fungi counts, and participating female older adults had higher lung function measures while homes of participating older adult males—in particular respondent homes 1 and 6—had higher fungal concentrations.

Finally, given the aforementioned findings, we attempted paired t -tests of means to examine potential seasonal differences of three of the specific fungal measures, PenAsp1mgb, FF2/FR1, and CCASUMFUNGI, but results were not statistically significant (data not shown). This was likely due in part to the pilot study's small sample.

DISCUSSION

This pilot study article represents cross-sectional analyses examining potential associations between indoor exposure—fungi counts during both the cooling and heating seasons of targeted fungi measured in kitchen, dining, and hallway floor areas of residences (estimate of exposure) of older adults with physician-diagnosed asthma and/or COPD—and disease status represented by FVC and FEV1. In summary, associations between higher concentrations of Cclad and FF2/FR1 fungi and lower FVC were observed in the cooling season and between higher concentrations of Enigr and PenAsp1mgb fungi and lower FVC in the heating season. We also found higher fungi counts (Cclad, Enigr, PenAsp1mgb, and FF2/FR1) in study homes were associated with lower lung function measures (FEV1 and FVC). Lung function measurements are known to be related to gender and age; gender stratification improved statistical analysis. In general, females had better lung function metrics than

TABLE 3.—Summary of results from generalized linear regression models examining selected lung function measures and selected measures of fungi (log-transformed data) in indoor floor surface dust among study participants in different seasons in Visalia, Tulare County, California.

Parameter	Cooling season					Heating season				
	Overall study sample		Stratified by gender ^a			Overall study sample		Stratified by Gender ^a		
	R^2	p -Value	R^2	Estimate	p -Value	R^2	p -Value	R^2	Estimate	p -Value
FEV1										
FF2/FR1 ^b	0.290	0.130	0.540	0.680	0.130	0.084	0.578	0.758	1.170	0.063*
CCAsumfungi ^c	0.134	0.332	0.380	0.823	0.174	0.032	0.736	0.773	1.264	0.052*
Cclad	0.130	0.350	0.380	0.810	0.170					
Enigr	0.080	0.460	0.390	0.900	0.130					
PenAsp1mgb	0.180	0.260	0.440	0.710	0.140					
FVC										
FF2/FR1 ^b	0.540	0.02*	0.720	0.520	0.100	0.014	0.822	0.524	0.895	0.171
CCAsumfungi ^c	0.325	0.109	0.427	0.478	0.340	0.028	0.750	0.617	0.992	0.121
Cclad	0.310	0.120	0.430	0.500	0.310					
Enigr	0.260	0.160	0.400	0.550	0.280					
PenAsp1mgb	0.240	0.180	0.440	0.560	0.210					

Notes: In the study data set, females had higher lung function measures, and males (in particular homes 1 and 6) had higher fungi measures. FEV1, forced expiratory volume in 1 second, measured in liters of air; FVC, forced vital capacity, measured in liters of air; qPCR, quantitative polymerase chain reaction.

^aIn these statistical analyses, females were compared to males.

^bThis is the universal fungal primer.

^cThis is the sum of four targeted allergenic taxa examined via qPCR in this study: Aaltr, Cclad, Enigr, and PenAsp1mgb.

p -value:

* $p < .10$.

males in this pilot study of older adults with chronic respiratory diseases (they were otherwise healthy and nonsmokers).

This pilot study's techniques have advantages and some new disadvantages (e.g., technical laboratory and human resources required) over standard available fungi or mold measurements conducted via settled dust and/or air sampling in the field for viable and/or nonviable spores (28, 37, 38). This type of work is challenging due to a lack of consistently used standard methods in the current literature (38). Our pilot study informs an alternative approach that is both rigorous and highly quantitative yet adaptable to community-based participatory research in homes in urban/suburban and rural agricultural settings.

Significant seasonal variation is known to occur in viable airborne fungi (1, 8, 24) and with fungi measured via qPCR (37, 39). Our study found high levels of FF2/FR1 and PenAsp1mgb in the cooling season in most homes and increased levels of Cclad and Enigr were also found in the heating season. Jacob et al. (24) reported higher levels of *Cladosporium* in wintertime household dust approximately tripled the risk of allergic sensitization in the children studied. However, in our pilot study, neither lung function measures across respondents nor selected fungi metrics based on paired *t*-tests of means statistically significantly varied with seasons. This may be in part due to the pilot study's small sample size, and in part because there was no systematic control of cleaning procedures practiced by study participants in their homes, for example, kitchen floors and bathrooms.

One respondent may have influenced some of the statistical trends observed in our study. More specifically, several apparent effects of fungi on lung function measures in this pilot study would not have been statistically significant without one respondent at home 6. This home had visible signs of water damage and mold (29). In one area there was a four square feet surface with visible mold, musty odor was detected, and there was a leak or drip contained by the kitchen basin. There was also missing or deteriorated caulk or seals and evidence of leaks or damage to the windows and/or surrounding frame structures. In addition, since there was no forced air or other mechanical heating, ventilation, and air conditioning system present, the house had less ventilation. Indeed, this respondent also exhibited poor lung function measures. Other researchers (40) reported individuals living in homes with confirmed areas of dampness had greater evidence of airflow obstruction than those living in dry homes. While it is known that increased moisture promotes the growth of fungi, other studies reported levels of cultured fungi were not associated with symptoms, and in one study no correlations were found between lung function parameters and any fungal exposure measure (16). More research with larger sample sizes, prospective designs, and disease-free controls is needed, in particular for emerging susceptible subpopulations defined by age, gender, and race/ethnicity.

Future indoor environmental health research on fungi should also further explore the exact reasons behind the importance of gender, in particular whether gender was more related to the exposure metric—household operation

and maintenance and resulting differences in levels of surface dust and fungi content—or to lung function and/or other physiological measures (e.g., lower body mass index, general health). Additionally, other social, economic, and demographic variables should be incorporated, for example, historical exposures to environmental tobacco smoke at work and at home in addition to smoking history, details of work history (other exposures), and so on. In this pilot study, subjects were currently nonsmokers and retired except one nonsmoker who still did some office work. Occupational exposure to higher levels of fungi and/or bacteria has been associated with allergic diseases, including asthma, among working adults (38, 41).

This study had known limitations. First, our pilot study did not have a control measurement of lung function for study participants without one or both chronic respiratory diseases of interest and/or corresponding to good indoor environments (housing condition). Dales et al. (7) reported an association with respiratory symptoms and levels of viable indoor fungi in dust, but not airborne fungi. In our pilot study, as a second limitation, we did not establish an exact quantitative relationship between airborne fungi and fungi in settled dust, but merely assumed a relationship existed. Nevertheless, older adults with reduced lung function who live in homes with higher fungal content are clearly at risk of exposure and thus of special public health concern. Third, although this pilot study focused on fungi, there is evidence of a significant role by house dust mites in causing respiratory allergy, specifically nasobronchial allergy, in sensitive individuals (23). The 2002 European Community Respiratory Health Survey, for example, observed associations among house mold, dust mite levels, and adult asthma (11). Exposure to dust mites is one risk factor for the development of asthma, and, like mold, may also exacerbate existing asthma (42–44). Fourth, while we accounted for intra-individual variability in field spirometry measures to the extent possible in this cross-sectional pilot study design, there is also known inter-individual variability; fungal sampling variability using methods employed in this pilot study was examined in detail in Yamamoto et al. (28). Finally, future field studies with more resources should have prospective designs with repeated measures across seasons and larger sample sizes across gender and race/ethnicity; this pilot study only included English-speaking white older adults, although we could stratify by gender and did not exclude race/ethnic groups.

CONCLUSIONS

This pilot study revealed some potential associations between targeted fungi in indoor home floor dust and selected lung function parameters from field spirometry measures of currently nonsmoking, otherwise healthy older adults (age 55 and older) with doctor-diagnosed asthma and/or COPD and of the influence of gender. The insight gained from this pilot study can more efficiently inform the design of larger scale prospective epidemiologic studies of older adults with chronic

respiratory diseases and of young children with asthma living in the same household. Future studies should collect both surface dust and air samples and possibly other allergens like dust mites and continue development of techniques to identify and quantify biological environmental asthma triggers at the species level. Additionally, these analyses may be used to justify better education for communities with respect to home maintenance as an important factor for lung health and to help motivate local agencies to provide support for home repairs among lower income lung impaired elderly individuals.

ACKNOWLEDGEMENTS

We gratefully acknowledge the members of the Tulare County Asthma Coalition who provided valuable input, suggested educational resources to provide for free at the home visits from local and state agencies and nonprofit organizations, and asked insightful questions during meetings in person or via conference call. We sincerely thank the participants, and their families, for their time and interest in our project. The funding source for this interdisciplinary pilot study was The Foundation of UMDNJ. We received in-kind support for various aspects of the project from UMDNJ-School of Public Health, San Joaquin Valley College (Visalia, CA), Yale University Department of Chemical and Environmental Engineering, and Tokai University (Kanagawa, Japan).

DECLARATION OF INTEREST

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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