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BRIEFCOMMUNICATION

Pilot study of mold in homes of asthmatic children in Taipei, Taiwan, using the Environmental Relative Moldiness Index

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Abstract The prevalence of asthma in Taiwan is one of the highest in Asia. Mold exposures have been linked to the development and exacerbation of asthma. A pilot study of mold populations in homes in Taipei, Taiwan, was conducted in the spring of 2014. Dust and air samples were collected from five homes with an asthmatic child and five from control homes. A combined, settled-dust sample was collected in the living room and bedroom in each home using a Swiffer^{TM} cloth. The dust (5 mg) was analyzed for 36 molds using a DNA-based analysis, mold-specific quantitative PCR, and mold contamination was estimated using the Environmental Relative Moldiness Index (ERMI) metric. The ERMI values were significantly ($p = 0.03$) greater in the homes of asthmatic children compared to the control homes, average 26.2 versus 17.4, respectively. *Aspergillus ochraceus* was found in significantly greater numbers in homes of asthmatic children compared to control homes. Air samples were collected in each home for 2 min (total 63 l of air) using the Burkard portable air sampler for agar plates fitted with malt extract agar plates. The plates were incubated at 25 °C for 5 days, and the resulting mold colonies were enumerated. Significantly higher total numbers of molds were cultured from the air in homes of asthmatics compared to control homes. Although this is a pilot study, it suggests that asthmatic children in Taipei, Taiwan, live in homes with significantly greater exposures to molds.

Keywords Mold · Dust · ERMI · Air · Culture

1 Introduction

Asthma is a chronic, inflammatory, respiratory disease that affects 300 million people worldwide (Masoli

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et al. 2004). In Taiwan, the prevalence of asthma increased from 5.07 % in 1985 to 11.9 % in 2007 (Hsieh and Shen 1988; Hwang et al. 2010). Many studies have shown that asthma is linked to mold in buildings, and these studies have been reviewed (IOM 2004; WHO 2009; Mendell et al. 2011; Quansah et al. 2012).

Recently, a prospective study showed that the odds of developing asthma doubled for infants exposed to homes with Environmental Relative Moldiness Index (ERMI) values above five (Reponen et al. 2011).

The ERMI scale was developed by the US Environmental Protection Agency (US EPA) in conjunction with the US Department of Housing and Urban Development to standardize the assessment of the mold contamination in US homes (Vesper et al. 2007) but has also been applied to homes in the tropical climate of Puerto Rico (Bolaños-Rosero et al. 2013) that is an island about on quarter the size of Taiwan. In Puerto Rico, the ERMI values inside of homes were significantly greater than outside.

For the ERMI analysis, a DNA-based technology, mold-specific quantitative PCR (MSQPCR), is used to measure the concentration of 36 indicator molds in settled-dust samples. Of the 36 indicator molds, 26 are "Group 1" species found in water-damaged homes and 10 "Group 2" species found in homes independent of water damage and that generally come from outdoors (Vesper 2011).

Recently, Chen et al. (2014) found a significant association between *Aspergillus/Penicillium* and basidiospores in classrooms of Taiwan schools and physician-diagnosed, current asthma. However, the mold contamination in the homes of these students was not measured. Our goal in this pilot study was to evaluate the mold populations in Taipei, Taiwan, homes of asthmatic children compared to control homes.

2 Materials and methods

In the spring of 2014, 10 families ($n = 10$) in Taipei, Taiwan, (Fig. 1) were recruited for a pilot study of mold contamination and asthma. Five homes with asthmatic children were designated as cases, and five were designated control homes. An inspection of each home was made to determine whether there was evidence of visible mold growth. Other home

Fig. 1 Location of Taipei in Taiwan

characteristics, including home type, home age, water damage at home, house plant, the number of walls with curtains, and carpet usage, were evaluated as well. Air and dust samples were obtained during the inspection.

Air samples were collected in each of the homes using the Burkard portable air sampler for agar plates (Burkard Scientific Ltd, Uxbridge, Middlesex, UK). There are two sampling sites at each home (i.e., the living room and the child room). A total of 63 l of air was sampled in 2 min onto malt extract agar (Oxoid, Basingstoke, UK) (prepared in the laboratory). The exposed plates were incubated for 5 days at 25 °C, and mold colonies were then enumerated on each plate by counting the number of colony-forming units (CFU) per cubic meter of air (m^3). All the fungal colonies were identified morphologically by an experienced microbiologist to the level possible by low-power microscopy (generally, to genus), and counts were recorded by colony type. Colony morphotypes were identified following previous literatures (Barnett and Hunter 1998; Germain 1996; Mallach 1982; Watanabe 2002).

Settled dust in each home was collected by wiping above floor-level surfaces in the living room and bedroom with a Swiffer™ sweeper cloth (Proctor and Gamble, Cincinnati, OH, USA). Sufficient dust was accumulated so that the cloth became visibly darkened. The dust samples were sent "blinded" to the US EPA for ERMI analysis. The dust was recovered from the Swiffer cloth by vacuuming with a MiTest™ sampler (Indoor Biotechnologies, Inc., Charlottesville, VA, USA) and then sieved through 300-μm pore nylon mesh (Gilson Company, Lewis Center, OH, USA). The DNA from each dust sample (5.0 ± 0.1 mg dust) was extracted, as previously described (Haugland et al. 2004), and the DNA was purified using the DNA-EZ kit (GeneRite, South Brunswick, NJ, USA).

Each of the 36 molds in the ERMI was quantified by MSQPCR, as previously described (Haugland et al. 2004). Briefly, the standard MSQPCR assays contained 12.5 μl of "Universal Master Mix" (Applied Biosystems Inc., Foster City, CA, USA), 1 μl of a mixture of forward and reverse primers at 25 μM each, 2.5 μl of a 400-nM TaqMan probe (Applied Biosystems Inc.), 2.5 μl of 2 mg/ml fraction V bovine serum albumin (Sigma Chemical, St. Louis, MO, USA), and 2.5 μl of DNA-free water (Cepheid, Sunnyvale, CA, USA). To this mix was added 5 μl of the DNA extract from the sample. All primer and probe sequences used in the assays are at the Web site: http://www.epa.gov/nerlcwww/moldtech.htm.

Average concentration and standard deviation (SD) of each of the 36 ERMI molds as well as the ERMI values from the homes of asthmatic children versus the control homes were compared using the Student's *t* test. The Wilcoxon rank-sum test was used to determine the significance in mold populations (total, *Cladosporium*, *Penicillium*, *Aspergillus,* and yeasts) quantified from culturing of the air samples in the homes. Correction for multiple comparisons of the species data was made using the Holms–Bonferroni test. Analyses were performed in SAS version 9.3 (SAS Institute, Cary, NC, USA) and R version 2.14 (R Foundation for Statistical Computing, Vienna, Austria).

3 Results

Table 1 shows the basic demographics of the 10 children and the main characteristics of their homes participated in this study. The average ERMI value measured in the homes of asthmatics ($26.2 \pm$ SD 6.82) was significantly greater ($p = 0.03$) than the average ERMI value in the control homes ($17.4 \pm$ SD 4.54). During the home inspections, only two of the homes were observed to be "visibly moldy." These two visibly moldy homes also had the highest ERMI values (37.71 and 26.62), and both were the homes of asthmatic children. The molds in these two homes with median values at least 100-fold greater than in the

Table 1 Basic demographics of the 10 children and the main characteristics of their homes

Index	Gender	Age	Asthma status	Home characteristics							ERMI
				Home type	Home age	Visible mold	Water damage	House plant	Curtain[a]	Carpet	
1	Boy	7	Yes	Apartment	28	No	No	No	3	No	20.49
2	Girl	7	Yes	Apartment	42	No	Yes	Yes	3	No	22.62
3	Boy	9	Yes	Apartment	25	No	No	No	3	No	23.39
4	Girl	8	Yes	Apartment	23	Yes	Yes	No	1	Yes	37.71
5	Boy	7	Yes	Apartment	30	Yes	Yes	No	3	No	26.62
6	Girl	7	No	Apartment	30	No	No	No	3	No	18.09
7	Boy	7	No	Apartment	30	No	No	No	1	Yes	10.92
8	Girl	8	No	Apartment	22	No	No	No	3	No	21.25
9	Boy	8	No	Apartment	27	No	No	No	1	No	21.78
10	Girl	9	No	Apartment	2	No	No	No	0	No	14.96

ERMI Environmental Relative Moldiness Index

[a] Number of walls with curtain at home

Table 2 Average concentration and standard deviation (SD) of each of the 36 Environmental Relative Moldiness Index molds in the homes of asthmatics versus controls

Mold	Asthmatic Average # cells/mg dust	SD	Control Average # cells/mg dust	SD	*t* test *p* value
Group 1					
Aspergillus flavus	4.2	2	3.6	2	0.63
Aspergillus fumigatus	0.8	0	0.2	0	0.04
Aspergillus niger	67.4	86	13.6	6	0.16
Aspergillus ochraceus	**9**	4	**1.4**	1	**0.00**
Aspergillus penicillioides	173,280	156,470	68,716	98,329	0.19
Aspergillus restrictus	1,639	2,025	313	560	0.15
Aspergillus sclerotiorum	10	21	1.4	3	0.33
Aspergillus sydowii	45.4	65	9.6	12	0.21
Aspergillus unguis	2,092	3,943	1,444	2,366	0.74
Aspergillus versicolor	2,155	4,125	9.2	5	0.23
Aureobasidium pullulans	123.2	162	38.6	25	0.23
Chaetomium globosum	17.2	23	6.4	8	0.30
Cladosporium sphaerospermum	23,660	34,280	663.4	1,201	0.13
Eurotium amstelodami	10,933.6	24,075	51	50	0.29
Paecilomyces variotii	2	2	0.2	0	0.05
Penicillium brevicompactum	11.2	7	2	2	0.01
Penicillium corylophilum	0	0	0.2	0	0.30
Penicillium crustosum	8.6	9	7	9	0.76
Penicillium purpurogenum	0.4	1	0.2	0	0.50
Penicillium spinulosum	0	0	0	0	ND
Penicillium variabile	4.6	7	1	1	0.21
Scopulariopsis brevicaulis	39.2	84	1	1	0.29
Scopulariopsis chartarum	5.6	9	1	1	0.22
Stachybotrys chartarum	3	5	2	4	0.71
Trichoderma viride	8.8	11	1.4	1	0.13
Wallemia sebi	8,904	8,212	1,490.6	2,808	0.06
Group 2		7		5	
Acremonium strictum	1.8	4	0.4	1	0.42
Alternaria alternata	16.4	18	4.4	3	0.14
Aspergillus ustus	0.6	1	0.2	0	0.19
Cladosporium cladosporioides 1	368	216	176.8	135	0.10
Cladosporium cladosporioides 2	743	1,597	1.8	1	0.28
Cladosporium herbarum	2.6	3	0.6	1	0.13
Epicoccum nigrum	14.6	16	3	2	0.10
Mucor racemosus	1.4	1	3.2	4	0.26
Penicillium chrysogenum 2	2.6	3	0.2	0	0.10
Rhizopus stolonifer	5.6	6	3.6	5	0.55

Species significantly different after corrections for multiple comparisons made using the Holms–Bonferroni test are bolded. (ND = not detectable because this mold was not detected in any sample)

Table 3 Average concentration and standard deviation (SD) of the culture-based analysis of the molds measured in air samples taken in asthmatic versus control homes

Cultured molds	Asthmatic mean (CFU/m^3)	SD	Control mean (CFU/m^3)	SD	Wilcoxon rank-sum test, p value
Total molds	2,426	3,104	310	166	0.012
Cladosporium sp.	506	527	63	59	0.060
Penicillium sp.	1,252	2,642	51	56	0.540
Yeast	115	175	52	58	0.596
Aspergillus sp.	319	544	31	17	0.525
Non-sporulating molds	365	227	131	57	0.080

The plates were incubated for 5 days at 25 °C, mold colonies were then enumerated on each plate, and the results are quantified as the number of colony-forming units (CFU) per cubic meter of air (m^3)

other eight homes were *Aspergillus restrictus*, *Aspergillus unguis*, *Aspergillus versicolor,* and *Eurotium amsteladomi* (data not given).

Penicillium spinulosum was the only mold, of the 36 ERMI molds, not detected in any of the samples from Taiwan (Table 2). Of the 36 ERMI molds, only the average population of *Aspergillus ochraceus* was significantly greater ($p < 0.001$) in the homes of asthmatic children compared to the control homes.

The mean total number of molds cultured from air samples collected in the homes of asthmatic children (2,426 CFU/m^3) was significantly greater (Wilcoxon rank-sum test, $p = 0.012$) than the mean total number cultured from control homes (310 CFU/m^3) (Table 3). On the other hand, none of the genera measured by culturing (*Cladosporium*, *Penicillium*, or *Aspergillus*) nor the yeasts was found to be significantly greater in the homes of asthmatic children compared to the control homes.

4 Discussion

In this pilot study, higher ERMI values and higher numbers of total cultured molds were found in the homes of asthmatic children in Taipei, Taiwan, compared to similar control homes. One advantage of using the ERMI metric compared to culturing was that we were able to quantify the concentrations of specific mold species. We showed that *A. ochraceus* was in significantly greater concentrations in the homes of asthmatic children compared to the controls (Table 2). On the other hand, the genera of molds measured by culturing did not show any significant differences, even for the genus *Aspergillus*. As Chen et al. (2014) noted in their study of mold and asthma in schools, the "species responsible for the development and attack of asthma remains inconsistent." Therefore, the identification and quantification of mold species in the homes of asthmatic children could be important in developing an understanding of the etiology of asthma.

Recently, Millien et al. (2013), in a mouse model of asthma, showed that some species of *Aspergillus* caused airway surface mycotic infections that resulted in chronic lung damage, including increases in the permeability of airway epithelial and vascular endothelial cells. These impacts to the lung initiated the cascade of events that resulted in asthma-like conditions in mice. Likewise, infants exposed to higher concentration of *A. ochraceus, A. unguis,* and *Penicillium variabile* were more likely to develop physician-diagnosed asthma at age seven (Reponen et al. 2012). Therefore, our finding of *A. ochraceus* in significantly greater populations in the homes of asthmatic children in Taiwan would be consistent with the Millien et al.'s hypothesis (Millien et al. 2013) and with the prospective study of childhood asthma (Reponen et al. 2012). However, no conclusions can be drawn from this small pilot study.

This pilot study does represent the first to use MSQPCR to identify and quantify mold species in Taiwan homes and to utilize the ERMI metric to describe the mold contamination. Although the ERMI metric was created for US housing, it has been applied to quantitatively describe mold contamination in other

tropical climates, including Puerto Rico and Singapore (Bolaños-Rosero et al. 2013; Yap et al. 2009). However, in tropical environments like Singapore, Puerto Rico, and Taiwan, the ERMI values are, in general, higher than typical in temperate climates. Therefore, it is likely that the ERMI scale for tropical countries would be improved by performing a random national sampling of homes, as was done to create the ERMI scale for the United States, and creating an ERMI scale just for these countries.

In spite of the limitations of this small study, the ERMI metric appears to be useful in identifying the mold-contaminated homes of asthmatic children in Taiwan. A greater understanding of the high prevalence of asthma in Taiwan might be developed by expanding this pilot study to the whole island.

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Conflict of interest The US Environmental Protection Agency (EPA) through its Office of Research and Development collaborated in the research described here. Although this work was reviewed by EPA and approved for publication, it may not necessarily reflect official EPA policy. Mention of trade names or commercial products does not constitute endorsement or recommendation by the EPA for use. Since MSQPCR technology is patented by the US EPA, the agency has a financial interest in its commercial use.

References

Barnett, H. L., & Hunter, B. B. (1998). *Illustrated genera of imperfect fungi* (4th ed.). St. Paul: APS Press.

Bolaños-Rosero, B., Betancourt, D., Dean, T., & Vesper, S. (2013). Pilot study of mold populations inside and outside of Puerto Rican residences. *Aerobiologia, 29*, 537–543.

Chen, C. H., Chao, H. J., Chan, C. C., Chen, B. Y., & Guo, Y. L. (2014). Current asthma in schoolchildren is related to fungal spores in classrooms. *Chest, 146*, 123–134.

Germain, G. S. (1996). *Identifying filamentous fungi: A clinical laboratory handbook*. Belmont: Star Publishing Company.

Haugland, R. A., Varma, M., Wymer, L. J., & Vesper, S. J. (2004). Quantitative PCR analysis of selected *Aspergillus*, *Penicillium* and *Paecilomyces* species. *Systematic and Applied Microbiology, 27*, 198–210.

Hsieh, K. H., & Shen, J. J. (1988). Prevalence of childhood asthma in Taipei, Taiwan, and other Asian Pacific countries. *Journal of Asthma, 25*, 73–82.

Hwang, C. Y., Chen, Y. J., Lin, M. W., Chen, T. J., Chu, S. Y., Chen, C. C., et al. (2010). Prevalence of atopic dermatitis, allergic rhinitis and asthma in Taiwan: A national study 2000 to 2007. *Acta Dermato-Venereologica, 90*, 589–594.

IOB (Institute of Medicine). (2004). *National academies of science. Damp indoor spaces and health*. Washington, D. C.: The National Academies Press.

Mallach, D. (1982). *Moulds: Their isolation cultivation and identification*. Toronto: University of Toronto Press.

Masoli, M., Fabian, D., Holt, S., & Beasley, R. (2004). The global burden of asthma: Executive summary of the GINA Dissemination Committee report. *Allergy, 59*, 469–478.

Mendell, M. J., Mirer, A. G., Cheung, K., Tong, M., & Douwes, J. (2011). Respiratory and allergic health effects of dampness, mold, and dampness-related agents: A review of the epidemiologic evidence. *Environmental Health Perspectives, 119*, 748–756.

Millien, V. O., Lu, W., Shaw, J., Yuan, X., Mak, G., Roberts, L., et al. (2013). Cleavage of fibrinogen by proteinases elicits allergic responses through Toll-like receptor 4. *Science, 341*(6147), 792–796.

Quansah, R., Jaakkola, M. S., Hugg, T. T., Heikkinen, S. A., & Jaakkola, J. J. (2012). Residential dampness and molds and the risk of developing asthma: A systematic review and meta-analysis. *PLoS One, 7*, e47526. doi:10.1371/journal.pone.0047526.

Reponen, T., Lockey, J., Bernstein, D. I., Vesper, S. J., Levin, L., Khurana Hershey, G. K., et al. (2012). Infant origins of childhood asthma associated with specific molds. *Journal of Allergy and Clinical Immunology, 130*(639–644), e635.

Reponen, T., Vesper, S., Levin, L., Johansson, E., Ryan, P., Burkle, J., et al. (2011). High environmental relative moldiness index during infancy as a predictor of asthma at 7 years of age. *Annals of Allergy, Asthma & Immunology, 107*, 120–126.

Vesper, S. (2011). Traditional mould analysis compared to a DNA-based method of mould analysis. *Critical Reviews in Microbiology, 37*, 15–24.

Vesper, S., McKinstry, C., Haugland, R. A., Wymer, L., Ashley, P., Cox, D., et al. (2007). Development of an Environmental Relative Moldiness Index for US homes. *Journal of Occupational and Environmental Medicine, 49*, 829–833.

Watanabe, T. (2002). *Pictorial atlas of soil and seed fungi: Morphologies of cultured fungi and key to species* (2nd ed.). Boca Raton: CRC Press LLC.

WHO Europe (World Health Organization Europe). (2009). *WHO guidelines for indoor air quality: Dampness and mould*. Copenhagen: WHO.

Yap, J., Toh, Z. A., Goh, V., Ng, L. C., & Vesper, S. (2009). Assessment of mold concentrations in Singapore shopping centers using mold-specific quantitative PCR (MSQPCR) analysis. *Indian Journal of Microbiology, 49*, 290–293.