Quantitative PCR analysis of molds in the dust from homes of asthmatic children in North Carolina

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Received 22nd March 2007, Accepted 22nd June 2007
First published as an Advance Article on the web 10th July 2007
DOI: 10.1039/b704359g

The vacuum bag (VB) dust from the homes of 19 asthmatic children in North Carolina (NC) was analyzed by mold specific quantitative PCR. These results were compared to the analysis of the VB dust from 176 homes in the HUD, American Healthy Home Survey of homes in the US. The Environmental Relative Moldiness Index (ERMI) was calculated for each of the homes. The mean and standard deviation (SD) of the ERMI values in the homes of the NC asthmatic children was 16.4 (6.77), compared to the HUD survey VB ERMI value mean and SD of 11.2 (6.72), and was significantly greater (*t*-test, $p = 0.003$) in the NC asthmatic children's homes. The molds *Chaetomium globosum*, *Aspergillus fumigatus*, and the *Eurotium* Group were the primary species in the NC homes of asthmatics, making the ERMI values significantly higher ($p < 0.02$ for each). Vacuum bag dust analysis may be a useful method for estimating the mold burden in a home.

Introduction

Asthma is the most common chronic disease of children in the United States (US)[1] and molds have been implicated in asthma's exacerbation.[2] In order to identify and quantify molds, we developed a DNA-based technology, mold specific quantitative PCR (MSQPCR), and a standardized method for collecting and analyzing dust samples for 36 molds.[3] In order to quantitatively describe the mold burden in a home, we developed the Environmental Relative Moldiness Index (ERMI).[3]

However, the cost of taking a standardized sample can be prohibitive. Also, some homeowners are hesitant to have strangers in their home to take the standardized dust sample. For these situations, we have examined the possibility of using the homeowner's vacuum bag (VB) as the dust sample source. We used this approach in analyzing a set of 19 homes of asthmatic children in North Carolina (NC) and compared the results to a set of 176 VB dust samples collected during the American Healthy Homes Survey completed by the Department of Housing and Urban Development (HUD).[3]

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Material and methods

Recruitment and eligibility

This study was approved by the University of North Carolina School of Medicine's Human Subjects Institutional Review Board and the US Environmental Protection Agency (EPA) Human Subjects Research official prior to study initiation. Persistent asthmatic children between the ages of 8 and 18 years were recruited from medical clinics and schools within 30 miles of Chapel Hill, NC. Each child was observed for six consecutive weeks between September of 2003 and June of 2004.[4] All eligible children received an onsite medical evaluation and skin prick allergy screening at the EPA Human Studies Facility (Chapel Hill, NC). This evaluation included focused medical and asthma histories.

Interested parents of children with asthma contacted our study recruitment office concerning eligibility requirements. Eligible participants were then scheduled for an enrollment screening and baseline clinical evaluation. Children were ineligible if their asthma symptoms were not persistent and severe, were an active smoker, pregnant, or had a medical history or underlying health problem that precluded participation (cystic fibrosis, viral bronchiolitis, bronchopulmonary dysplasia, heart disease, vocal cord dysfunction, laryngotracheomalacia, tracheal stenosis, bronchostenosis). If the screened child was found to have persistent asthma upon examination, they were enrolled into the six week panel study. Each child provided their informed assent and a parent or legal guardian provided their informed consent prior to study

enrollment. Once a child was enrolled, a request was made for the parents to bring in their current VB for mold analysis.

Dust analysis and ERMI calculation

Vacuum cleaner dusts (bagged or bagless) were collected in polypropylene zip top collection bags. Samples were gamma irradiated until each received a total minimum dose of 2.5 millirads. Individual dust samples were transferred from the vacuum cleaner bags (or polypropylene zip top bags, in the case of bagless vacuum samples) into a clean, sieve stacked on top of a 150 micron sieve equipped with a clean stainless steel bottom collection unit and lid. This assembly was placed in a Syntron Jogger J-1 Sieve Shaker (Syntron, Bioresearch, Carlsbad, CA, USA) and the unit was operated at least 75% sieve energy for 30 to 45 min.

The Environmental Relative Moldiness Index (ERMI) values are based on the analysis of 5 mg of the sample dust analyzed by MSQPCR. MSQPCR analyses were performed on the sieved dust, as previously described.[5–10] All primer and probe sequences, as well as known species comprising the assay groups, were published at the website: http://www.epa.gov/nerlcwww/moldtech.htm. The ERMI is calculated by taking the sum of the logs of the concentrations of the Group 1 species and subtracting from that the sum of the log of the concentrations of Group 2 species.[3] The ERMI is a single numeric value that represents the mold burden in the home.[3]

Statistical analyses

In general, mold concentration data analyzed with MSQPCR, having a minimum detection limit of 1 cell equivalent per mg dust, were treated as left-censored data with appropriate statistical methods applied.[11] Standard, non-parametric statistical methods were used for analyses of MSQPCR data having a single minimum detection limit.

The distributions of ERMI values between the US and NC sample populations were statistically compared using the Student *t*-test and graphically shown using box plots. (Cleveland[12] provides a detailed explanation of box plots including their uses and interpretations.) Comparisons were made between the NC and US VB samples on the basis of sample composition and mold spore concentration. For sample composition comparisons, each of the 36 species' concentration was expressed as the proportion of total spore count in home samples. The sums across all 36 species proportions were equal to one (1) for each home sample. Comparisons on this basis were on a standardized scale, and focused on the representation of each mold species in the total sample, and its relative over-representation in the sample populations. Statistical differences for sample composition and spore concentration were based on the Wilcoxon rank sum test, since these data were derived from left-censored spore concentrations used in MSQPCR analyses.

Results

North Carolina demographics

All children in this study were atopic, *i.e.* tested positive by skin prick test. The mean age of the asthmatic children was 11.8 years, approximately equal numbers of boys and girls and mostly Caucasian (90%). Over 85% of the children had mild persistent to severe persistent asthma. The families lived primarily (85%) in single family homes of which 55% were between 11 and 30 years old. Self-reported visible mold was described in only 20% of the residences.

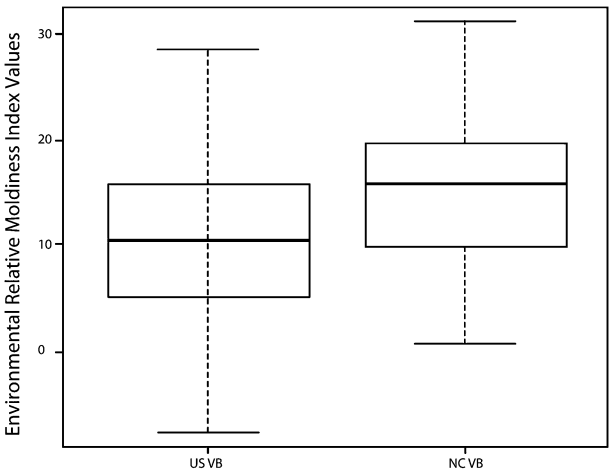

Fig. 1 Box plot of ERMI values for vacuum bag dust from homes with an asthmatic child in North Carolina (NCVB) ($n = 19$) *versus* the vacuum bag dust from US homes (USVB) ($n = 176$). The two distributions are significantly different (Student *t*-test, $p = 0.003$).

Molds in dust based on MSQPCR analysis

The box plots comparing the NC ERMI values and US ERMI values are shown in Fig. 1. The mean and standard deviation (SD) of the ERMI values in the homes of the NC asthmatic children was 16.4 (6.77). This compares to the HUD survey vacuum cleaner ERMI value mean and SD of 11.2 (6.72). In the NC asthmatic homes, 74% had ERMI values above the mean of the HUD Survey ERMI values. The overall distribution of ERMI values was significantly greater (Student's *t*-test, $p = 0.003$) in the 19 asthmatic homes than in the national survey of US homes.

Table 1 shows the concentrations of each of the 36 species in the NC asthmatics' homes and in the survey of US homes. There are 12 Group 1 and 6 Group 2 species in significantly higher concentrations in the NC homes ($p < 0.02$ by the Wilcoxon rank sum test).

Table 2 shows differences in the relative composition of the two sample populations. The mean percentages of each of the 36 mold species are compared between the NC asthmatics' homes and the survey of US homes. Comparison of relative sample composition was done on a standardized scale (0 to 1) to test how each of the 36 species is proportionally represented in the two sample populations. Of the species that are significantly different by the Wilcoxon rank sum test ($p < 0.02$), only *Chaetomium globosum*, *Aspergillus fumigatus*, and the *Eurotium* Group showed higher ratios (20.90, 1.57 and 2.98, respectively) in the VB dust samples from NC asthmatics' homes compared to US homes (Table 2).Discussion

In spite of the fact that only 20% of the NC homeowners reported having visible mold in their homes, 74% of the NC homes actually had mold burdens higher than the US median.

Table 1 Cell concentrations expressed as cell equivalents (CE) of each mold species in the North Carolina (NC) study homes compared to the United States (US). The Wilcoxon rank sum test results are shown with *p* values adjusted for multiple comparisons. Mold species in bold are in significantly ($p < 0.02$) greater numbers in the NC study homes compared to the US

Mold species	NC study CE mg^{-1} dust	US survey CE mg^{-1} dust	Rank sum test *p*-value
Group 1			
Aspergillus fumigatus[a]	**44**	**7**	**<0.001**
***Eurotium* Group**[b]	**5362**	**730**	**<0.001**
Chaetomium globosum	**2493**	**12**	**<0.001**
Paecilomyces variotii	**72**	**18**	**<0.001**
Penicillium variabile	**78**	**12**	**<0.001**
Cladosporium sphaerospermum	**786**	**146**	**<0.001**
Stachybotrys chartarum	**19**	**3**	**<0.001**
Trichoderma viride[c]	**62**	**14**	**<0.001**
Aspergillus ochraceus[d]	7	445	0.001
Penicillium brevicompactum	**126**	**46**	**0.002**
Scopulariopsis chartarum	**35**	**5**	**0.002**
Penicillium spinulosum[e]	**91**	**15**	**0.010**
Scopulariopsis brevicaulis	**12**	**6**	**0.015**
Aspergillus unguis	59	21	0.037
Wallemia sebi	159 334	570	0.042
Aspergillus versicolor	38 637	65	0.063
Aspergillus sclerotiorum	18	28	0.123
Aureobasidium pullulans	1495	1795	0.666
Aspergillus restrictus[f]	2342	20	0.740
Aspergillus niger[g]	15	38	0.908
Aspergillus flavus[h]	4	4	1.000
Aspergillus penicillioides	5207	3331	1.000
Aspergillus sydowii	431	8	1.000
Penicillium corylophilum	32	12	1.000
Penicillium Group 2[i]	19	27	1.000
Penicillium purpurogenum	3	4	1.000
Group 2			
Rhizopus stolonifer	**31**	**1**	**<0.001**
Cladosporium herbarum	**1337**	**109**	**<0.001**
***Cladosporium cladosporioides* (Type 2)**	**159**	**15**	**<0.001**
Mucor racemosus[j]	**271**	**24**	**<0.001**
***Penicillium chrysogenum* (Type 2)**[k]	**530**	**44**	**<0.001**
Alternaria alternata	**197**	**29**	**0.011**
Acremonium strictum	23	16	0.249
Aspergillus ustus	10	11	0.767
Cladosporium cladosporioides (Type 1)	1887	521	1.000
Epicoccum nigrum	86 553	1626	1.000

[a] Includes *A. fumigatus* and *Neosartorya fischeri*. [b] Includes *E. amstelodami*, *E. chevalieri*, *E. herbariorum*, *E. rubrum* and *E. repens*. [c] Includes *T. viride*, *T. atroviride* and *T. koningii*. [d] Includes *A. ochraceus* and *A. ostianus*. [e] Includes *P. spinulosum*, *P. glabrum*, *P. lividum*, *P. pupurescens* and *P. thomii*. [f] Includes *A. restrictus*, *A. caesillus* and *A. conicus*. [g] Includes *A. niger*, *A. foetidus* and *A. pheonicis*. [h] Includes *A. flavus* and *A. oryzae*. [i] Includes *P. crustosum*, *P. camembertii*, *P. commune*, *P. echinulatum* and *P. solitum*. [j] Includes *M. amphibiorum*, *M. circinelloides*, *M. hiemalis*, *M. indicus*, *M. mucedo*, *M. racemosus*, *M. ramosissimus*, *R. azygosporus*, *R. homothallicus*, *R. microsporus*, *R. oligosporus* and *R. oryzae*. [k] This is dominant subgroup of species.

Mold growth, or the extent of mold growth, is not always obvious in a home[3] but the ERMI can help to describe the mold burden. The use of the ERMI scale has been shown to be useful in characterizing asthma and mold associations.

For example, higher ERMI values were found in water-damaged homes of asthmatic children in Cleveland, OH[13] and pre-asthma symptoms were associated with higher ERMI values in a prospective study of atopic infants.[14] The ERMI values were actually more predictive of a negative health outcome than the home mold inspection.

By using the ERMI scale, we have seen that indoor molds in homes across the US are very similar.[3] This should not be a surprise since the temperatures in US homes are kept generally about the same and the food sources are similar. Water availability is the major variable. Therefore, we can compare dust samples in a set of control homes across the US to homes in North Carolina.

In some epidemiological studies, it is not always possible to obtain the preferred, standardized dust sample. Even so, analyses of the VB dust was useful in demonstrating that the mold burden in these homes of NC children with asthma were significantly higher than the VB dust from a random selection of US homes.

Many species of molds were found in higher numbers in the dust samples from asthmatic's homes (Table 1). However, looking at the relative percentage of each species in the samples allowed us to determine which species primarily contributed to the difference in the samples. The Group 1 species, *Chaetomium globosum*, *Aspergillus fumigatus*, and the *Eurotium* Group, were found to be the major contributors to the higher ERMI values in the homes of NC asthmatic children, compared to homes in the rest of the US.

In an earlier study of the water-damaged homes of asthmatic children in Cleveland, some Group 1 mold species were

Table 2 Differences in the relative composition of mold populations between the NC asthmatics' homes and the survey of US homes based on the mean percentages of each of 36 mold species. The Wilcoxon rank sum test results are shown with *p*-values adjusted for multiple comparisons. Mold species in bold are shown to be significantly ($p < 0.02$) larger sample components in the NC study homes compared to the US

Mold species	NC percentage	US survey percentage	Ratio NC : US	Rank sum *p*-value
Group 1				
Aspergillus ochraceus	0.03	4.45	0.01	<0.001
Aspergillus unguis	0.02	0.49	0.05	<0.001
Penicillium purpurogenum	0.02	0.10	0.19	0.003
Chaetomium globosum	**6.98**	**0.33**	**20.90**	**0.013**
Penicillium crustosum	0.19	0.48	0.38	0.016
Aspergillus flavus	0.02	0.16	0.14	0.018
Aspergillus fumigatus	**0.29**	**0.19**	**1.57**	**0.018**
Aureobasidium pullulans	11.51	24.63	0.47	0.018
***Eurotium* Group**	**20.09**	**6.74**	**2.98**	**0.018**
Aspergillus penicillioides	3.87	17.97	0.22	0.034
Penicillium corylophilum	0.24	0.36	0.69	0.040
Aspergillus sydowii	0.05	0.14	0.36	0.043
Aspergillus niger	0.11	1.03	0.11	0.069
Aspergillus sclerotiorum	0.10	0.43	0.24	0.325
Scopulariopsis chartarum	0.09	0.08	1.05	0.442
Scopulariopsis brevicaulis	0.06	0.14	0.43	0.455
Cladosporium sphaerospermum	3.43	2.22	1.55	0.570
Stachybotrys chartarum	0.09	0.09	0.96	0.588
Penicillium variabile	0.47	0.26	1.80	0.628
Paecilomyces variotii	0.41	0.44	0.95	0.879
Aspergillus restrictus	3.10	0.47	6.63	1.000
Aspergillus versicolor	3.09	1.05	2.93	1.000
Penicillium brevicompactum	0.76	0.95	0.80	1.000
Penicillium spinulosum	0.55	0.42	1.30	1.000
Trichoderma viride	0.34	0.36	0.94	1.000
Wallemia sebi	13.24	4.45	2.97	1.000
Group 2				
Cladosporium herbarum	5.90	3.07	1.92	0.040
Epicoccum nigrum	5.97	17.57	0.34	0.051
Mucor racemosus	1.54	0.56	2.76	0.062
Cladosporium cladosporioides (Type 2)	0.36	0.46	0.77	0.253
Aspergillus ustus	0.08	0.35	0.23	0.296
Penicillium chrysogenum	1.96	1.03	1.90	0.576
Acremonium strictum	0.12	0.55	0.21	0.628
Cladosporium cladosporioides (Type 1)	14.11	7.31	1.93	0.628
Alternaria alternata	0.63	0.62	1.02	1.000
Rhizopus stolonifer	0.20	0.05	4.02	1.000

in significantly greater concentrations than the control homes.[13] When the water damage and mold were removed from Cleveland homes, the asthmatic children who had been living in those homes had a ten-fold reduction in their need for emergency room visits or hospitalizations for their asthma.[14]

In a prospective study of infants, Group 1 mold species were significantly higher in concentration in homes where infants were found to be more likely to develop wheeze and rhinitis.[15] Similarly, in this present study, the concentrations of some Group 1 molds were associated with the homes of asthmatic children in NC. However, it must be emphasized that these results only demonstrate an association and not a proof of causation. Rather, the Group 1 molds are indicators of water-damage and the ERMI is a useful tool to quantify these indicator species. If it is not possible to take the standard dust sample, then VB dust may still be useful in understanding the mold burden in an asthmatic's home. Many additional epidemiological studies are in progress.

Disclaimer

The U.S. Environmental Protection Agency (EPA) through its Office of Research and Development, and Housing and Urban Development (HUD) funded and collaborated in the research described here. It has been subjected to each Agency's peer review and has been approved as an EPA publication. 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.

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