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Short Communication

## The level of submicron fungal fragments in homes with asthmatic children

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## ABSTRACT

**Objectives:** Much scientific evidence indicates a positive association between moldy environments and respiratory illnesses and/or symptoms (e.g., asthma). Recently, submicron fungal fragments ( $< 1.0 \mu\text{m}$ ) have been suggested as a potential contributor to adverse health effects due to their biological composition (e.g., antigens, mycotoxins, and (1,3)- $\beta$ -D-glucan) as well as their small size. However, the contribution of exposure to fine fungal particles on adverse health outcomes has been poorly characterized, particularly in homes with asthmatic children. We characterized the airborne level of smaller-sized fungal particles between homes with and without asthmatic children.

**Methods:** We visited 29 homes with ( $n=15$ ) and without ( $n=14$ ) an asthmatic child and sampled submicron fungal fragments in a living room and child's bedroom, along with outdoor sampling, using the NIOSH two-stage sampler. (1,3)- $\beta$ -D-glucan of fungal fragments analyzed by *Limulus Amebocyte* lysate assay (LAL) was used for quantifying their exposure.

**Results:** Overall, the geometric mean (GM) concentration of (1,3)- $\beta$ -D-glucan in submicron fungal fragments in indoor air was two-fold higher in homes with asthmatic children ( $50.9 \text{ pg}/\text{m}^3$ ) compared to homes with non-asthmatic children ( $26.7 \text{ pg}/\text{m}^3$ ) ( $P < 0.001$ ). The GM concentration of these particles in child's bedroom in homes with an asthmatic child ( $66.1 \text{ pg}/\text{m}^3$ ) was about three times higher than that in homes with non-asthmatic children ( $23.0 \text{ pg}/\text{m}^3$ ) ( $P < 0.001$ ). The relative humidity had a negative correlation with the concentration of (1,3)- $\beta$ -D-glucan in submicron fungal fragments (Pearson coefficient =  $-0.257$ ,  $P=0.046$ ).

**Conclusions:** Our findings indicate that homes with asthmatic children have a higher concentration of submicron fungal fragments compared to homes with non-asthmatic children. A greater exposure to smaller-sized fungal particles may occur in homes with an asthmatic child as relative humidity decreases. The very careful control of relative humidity in indoor air is necessary for reducing exposure to fine fungal particles and inhibiting the growth of microorganisms in homes with allergic diseases.

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### 1. Introduction

Many epidemiological studies and a recent review by the WHO showed that increased exposure to mold attributed to water-damaged or damp conditions in buildings could causally contribute to exacerbation of asthma symptoms (Afshari et al., 2009; Antova et al., 2008; Carpenter, 2004; Fisk et al., 2007; Institute of Medicine, 2004). The development of asthma could be attributed to allergic responses linked with exposure to mold (Fung and Hughson, 2003; Robbins et al., 2000), but the causal mechanism is still unclear. Therefore, a proper exposure assessment for mold in

indoor environments is necessary, and a tailor-made strategy for prevention and alleviation of asthma symptoms should be prepared based on the results of a proper assessment of indoor mold.

The culture method followed by air sampling for indoor mold has been used in many previous studies to investigate the association of exposure to mold with the development or exacerbation of asthma (Afshari et al., 2009; Institute of Medicine, 2004). However, the limitations of this method due to short sampling times, the different growth rates by types of mold, and culturability dependence on media make it difficult to determine the cause-and-effect relationship of mold exposure on disease outcomes (Reponen et al., 2011; Vesper et al., 2009, 2007). In recent years, fine fungal particles of less than  $1 \mu\text{m}$  (hereinafter 'submicron fungal fragments'), which contain fungal allergens, mycotoxins, and (1,3)- $\beta$ -D-glucan, have been suggested as a potential

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factor affecting the exacerbation of disease symptoms (Reponen et al., 2007; Seo et al., 2008, 2009). The submicron fungal fragments can stay airborne longer than fungal spores, which are larger, and can penetrate deeply into lungs and be deposited due to their small aerodynamic diameter. A previous study has shown that submicron particles attribute to broken spores and hyphae of *Stachybotrys chartarum* may be deposited at a rate 230-fold higher than intact airborne spores (Cho et al., 2005). In addition, exposure to airborne fine particles has been linked with adverse health effects on the respiratory and cardiac responses. In particular, the number concentrations of ultrafine particles ( $< 0.1 \mu\text{m}$ ), rather than the mass concentrations of these particles, have been strongly associated with adverse health effects (Penttinen et al., 2001; Peters et al., 1997; Von Klot et al., 2002). For this reason, a greater health impact from submicron fungal fragments might be expected due to their smaller size and higher number concentration. However, the contribution of submicron fungal fragments including debris of spores and hyphae on health are poorly characterized.

In this study, we evaluated and compared the level of submicron fungal fragments expressed as the concentration of (1,3)- $\beta$ -D-glucan between homes with and without asthmatic children. In addition, the effect of physical factors in indoor air such as temperature and humidity on the concentration of submicron fungal fragments was evaluated.

## 2. Methods

### 2.1. Study subjects

We selected 15 homes with asthmatic children shown to be sensitized to mold only by skin prick tests among the childhood asthmatics registered at the Environmental Health Center for Asthma, Korea University General Hospital, during 2010 and 2011. We also chose 14 homes with non-asthmatic children determined by a physician after visiting the Pediatrics Clinic, Korea University General Hospital, selected to have similar ages, genders, the number of people residing, and size of dwellings as shown in Table 1. None of the non-asthmatic children were also sensitized with any allergen of 18 common aeroallergens used for the skin prick test. This study was approved by the institutional review board of the Korea University Anam Hospital (No. ED07111).

### 2.2. Exposure assessment

We used a questionnaire for collecting demographic information of children and data of characteristics of dwellings: types and size of dwellings, living level (ground floor or higher), house age, and the presence of visible mold or water stains on the wall or ceilings. Indoor investigations of dwellings were also performed by trained researchers, and the procedures of exposure assessment are described below in detail.

#### 2.2.1. Air sampling for submicron fungal fragments and analysis

Air samples for submicron fungal fragments were collected using the NIOSH two-stage sampler (Lindsley et al., 2006) as described in detail elsewhere (Seo et al., 2008). Briefly, each NIOSH two-stage sampler was loaded with a 37 mm gamma-irradiated polycarbonate filter with a pore size of  $0.8 \mu\text{m}$  (SKC Inc., Eighty Four, PA, USA) and connected to a pump (Gillian 5000; Sensidyne, FL, USA). The samplers were placed in the living room and child's bedroom (indoor) and balcony (outdoor) of the visited homes (total number of samples: 3 samples (indoor+outdoor)/home  $\times$  29 homes = 87 samples). Sampling was performed for about 7–8 h depending on the overall concentrations of airborne particles determined by an optical particle counter (Model 1.108, Grimm Technologies, Inc., Douglasville, GA, USA) to avoid spores to bounce into the filter (Seo et al., 2007). For quality control field blank samples were installed in the living room and balcony. After the sampling, the filter cassette was covered with aluminum foil, placed on ice, and then stored at  $4^\circ\text{C}$  until analysis. To minimize the effect of external factors such as rain and anthropogenic disturbances, the sampling was carried out approximately from 8:00 am to 4:00 pm during September and October of 2012 under empty house and closed conditions of all the windows. Also, all parents were asked not to clean their home the day before and on the day of sampling to avoid the release of fungal spore and fine particles into indoor air attributed to different cleaning practices.

**Table 1**

Characteristics of dwellings and demographic information of asthmatic and non-asthmatic children.

	Group with asthmatic children (case)	Group with non-asthmatic children (control)
Number of subjects	15	14
Gender (boy/girl)	8/7	6/8
Age (years) (mean $\pm$ SD <sup>a</sup> )	8.1 $\pm$ 1.6	8.5 $\pm$ 1.3
Size of dwellings (mean $\pm$ SD <sup>a</sup> ) (m <sup>2</sup> )	79.2 $\pm$ 9.2	83.1 $\pm$ 7.3
House age		
< 1990	4	2
> 1990	11	12
Living level		
Ground floor	6	3
Next floor	9	11
Types of house		
Apartment	14	12
Row house	1	2
Visible mold or water stains		
Yes	3	5
No	12	9

<sup>a</sup> SD; standard deviation.

The concentration of submicron fungal fragments was estimated by analyzing (1,3)- $\beta$ -D-glucan, which exists in fungal cell walls and is known to cause severe inflammation in the respiratory system. The kinetic chromogenic LAL (*Limulus Amebocyte* lysate) analysis (Glucatel; Associates of Cape Cod, East Falmouth, MA, USA) for measuring (1,3)- $\beta$ -D-glucan has been used in many previous studies (Iossifova et al., 2009; Reponen et al., 2007; Seo et al., 2007, 2008, 2009). Briefly, the filter from the cassette was transferred to a 15 ml conical tube (Fisher Scientific, Pittsburgh, PA, USA), and submicron fungal fragments were extracted using 5 ml of PBS solution (Fisher Scientific, Pittsburgh, PA, USA) with the use of a vortexer (Model 231; Fisher Scientific, Pittsburgh, PA, USA) and a sonicator (Model FS20; Fisher Scientific Inc., Pittsburgh, PA, USA). A 1.0 ml-aliquot of the extracted solution was used to make stained glass slides for microscopic examination using a light microscope (Eclipse Ci; Nikon Corporation, Tokyo, Japan) for checking the absence of fungal spores. The field sampling was performed again if the fungal spores were observed on the stained filter, and the solution without fungal spores only was used for the (1,3)- $\beta$ -D-glucan analysis. To make (1,3)- $\beta$ -D-glucan with a tertiary structure water-soluble, 0.5 ml of 0.6 M NaOH was added to 0.5 ml of the extracted solution, and it was agitated for 1 h using a mechanical agitator (Model 75 Wrist-action shaker; Burrell Scientific, Pittsburgh, PA, USA). Following the agitation, 50  $\mu\text{l}$  of (1,3)- $\beta$ -D-glucan lysate was added to 25  $\mu\text{l}$  of the solution mixed with NaOH, and the concentration of (1,3)- $\beta$ -D-glucan was measured for about 150 min using an absorbance microplate reader (ELx808TM; Bio-Tek Instruments Inc., Winooski, VT, USA). The analysis result was expressed in pg/ml, and by estimating the total sampling time, it was converted to pg/m<sup>3</sup>.

#### 2.2.2. Sampling for airborne mold and analysis

We sampled airborne mold in the living room and child's bedroom separately using a one-stage Andersen sampler (Andersen Instruments, Atlanta, GA, USA). Air sampling was performed for 10 min using a vacuum pump (Model no. 1531-107B-G289X, Gast Manufacturing, Benton Harbor, MI, USA) at a flow rate of 28.98 LPM. We used malt extract agar supplemented with cycloheximide (0.5 g/L) (Sigma-Aldrich, St. Louis, MO, USA) to inhibit the growth of bacteria from homes. The colony numbers were counted on the plates to determine the concentrations of airborne mold after a 4-day incubation at 20–25  $^\circ\text{C}$ . Each field blank for mold at each home was taken and tested for growth. Nearly all of the blank samples had no or one colony, so sample counts were adjusted for the reading of blank samples. The concentrations were finally expressed as the corrected colony numbers followed by the positive-hole correction per air volume (CFU/m<sup>3</sup>) (Macher, 1989). In addition, the temperature and relative humidity in each living room and child's bedroom were monitored using a portable thermo-hygrometer (GrayWolf Sensing Solution, Tuamgraney, Co., Clare, Ireland).

### 2.3. Statistical analysis

The concentration of (1,3)- $\beta$ -D-glucan in submicron fungal fragments showed a right-skewed distribution in normality tests. The geometric mean (GM) was used for reporting the results in this study. The Wilcoxon signed-rank test was performed to compare the indoor and outdoor concentrations of (1,3)- $\beta$ -D-glucan in submicron fungal fragments between homes with and without asthmatic children. Also, the Wilcoxon matched-paired signed-rank test was performed to compare the concentrations between the living room and child's bedroom. The

Pearson correlation analysis was performed to investigate associations among indoor temperature, relative humidity, the concentration of (1,3)- $\beta$ -D-glucan in submicron fungal fragments, and the concentration of airborne mold. The association between the concentration of (1,3)- $\beta$ -D-glucan in submicron fungal fragments and each independent variable (temperature, relative humidity, the concentration of airborne mold, house age, living level, types of house, and the presence of visible mold or water stains) was evaluated by the multivariate regression. All data were analyzed using the SAS<sup>®</sup> program (version 9.1; SAS Inc., Cary, NC), and a significance level ( $\alpha$ ) of 5% was applied unless otherwise indicated.

### 3. Results

#### 3.1. Demographic characteristics of asthmatic group and control group

The proportion of boys were slightly higher in the asthmatic children (53%) compared to the non-asthmatic children, 53% of whom were girls (Table 1). The average ages of the two groups were 8.1 years for the asthmatic children and 8.5 years for the non-asthmatic children, which were not significantly different ( $P=0.652$ ). Most of subjects lived in apartments built after the 1990s in Seongbuk-gu, Seoul. The mean size of the dwellings was 79 m<sup>2</sup> for the asthmatic children and 83 m<sup>2</sup> for non-asthmatic children. More visible molds or water stains on the walls or ceilings were observed in homes with non-asthmatic children (36%) than in homes with asthmatic children (20%), but significant difference was not found ( $P=0.349$ ).

#### 3.2. Concentration of (1,3)- $\beta$ -D-glucan in submicron fungal fragments

Fig. 1 shows the indoor and outdoor concentrations of (1,3)- $\beta$ -D-glucan in submicron fungal fragments in both homes with and without asthmatic children. The geometric mean (GM) concentration of (1,3)- $\beta$ -D-glucan in submicron fungal fragments in indoor at homes with an asthmatic child was 50.9 pg/m<sup>3</sup> (GM  $\pm$  SD; 30.4–85.3). This value was about two times higher than that at homes with non-asthmatic children (26.7 pg/m<sup>3</sup> (15.6–45.6)), and the difference was statistically significant ( $P < 0.001$ ). The average outdoor concentrations for homes with and without an asthmatic child were 19.2 (13.4–27.5) pg/m<sup>3</sup> and 21.3 (13.3–34.2) pg/m<sup>3</sup>, respectively, and there was no significant difference. The average indoor/outdoor (I/O) ratio for the concentrations of (1,3)- $\beta$ -D-glucan in submicron fungal fragments at homes with asthmatic

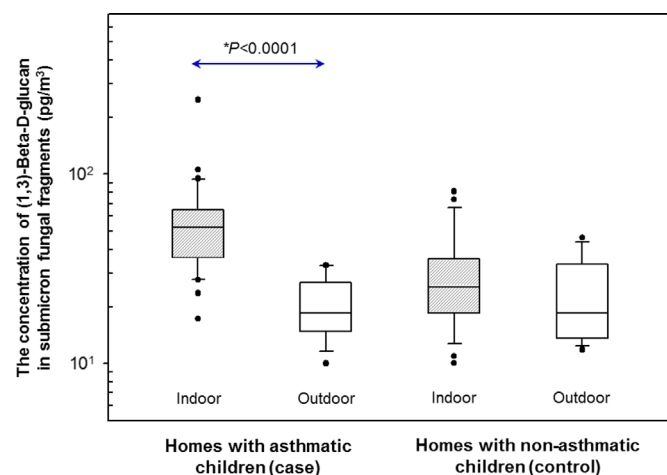


Fig. 1. The concentration of (1,3)- $\beta$ -D-glucan in submicron fungal fragments in indoor and outdoor of homes with and without asthmatic children; each box plot indicates an interquartile range (IQR) with median, upper and lower whiskers; upper and lower boundaries (3rd quartile/1st quartile).

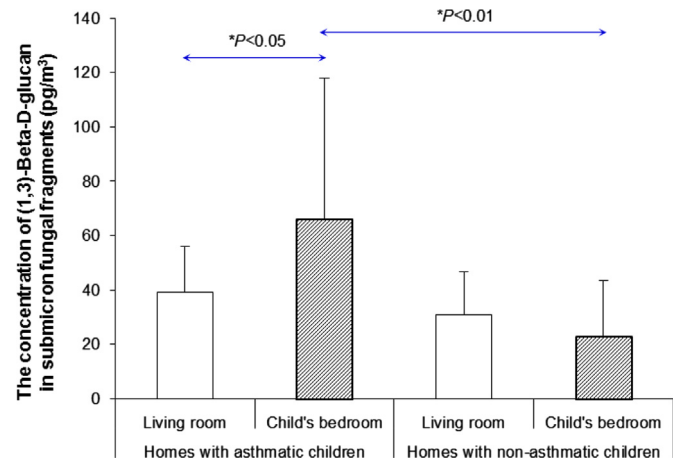


Fig. 2. The comparison of the geometric mean concentration of (1,3)- $\beta$ -D-glucan in submicron fungal fragments between a living room and child's bedroom in homes with and without asthmatic children. Histograms and error bars present the geometric mean (GM) and standard deviation, respectively.

children was 3.2, and this difference was significant ( $P < 0.001$ ). In contrast, the average I/O ratio in homes with non-asthmatic child was about 1.5 times, and this difference was not significant.

The GM concentration of (1,3)- $\beta$ -D-glucan in submicron fungal fragments in the bedrooms of asthmatic children (66.1 pg/m<sup>3</sup>) was significantly higher than that in bedrooms of non-asthmatic children (23.0 pg/m<sup>3</sup>) ( $P=0.008$ ), while no significant difference was observed between the living rooms (Fig. 2). The concentration in the child's bedroom was about 1.7 times higher than that in the living room (39.3 pg/m<sup>3</sup>) for asthmatic children, which was significant ( $P=0.031$ ). The GM concentration in the living room in homes with non-asthmatic child was 11.8 pg/m<sup>3</sup>, which was not significantly different from that in child's bedroom ( $P=0.441$ ).

#### 3.3. Pearson correlation coefficient and univariate analyses

The results of the correlation analysis among the concentration of (1,3)- $\beta$ -D-glucan in submicron fungal fragments, the concentration of airborne mold, temperature, and relative humidity are shown in Table 2. Except for relative humidity, the variables generally had no correlation with the concentration of (1,3)- $\beta$ -D-glucan in submicron fungal fragments. However, a negative correlation of relative humidity with the concentration of (1,3)- $\beta$ -D-glucan in submicron fungal fragments was observed to be significant (Pearson coefficient =  $-0.257$ ,  $P=0.046$ ). The additional multivariate analysis showed that the concentration of (1,3)- $\beta$ -D-glucan in submicron fungal fragments decreases by 0.82 pg/m<sup>3</sup> as 1% of humidity increases ( $aR^2=0.071$ ,  $P=0.004$ ; Fig. 3).

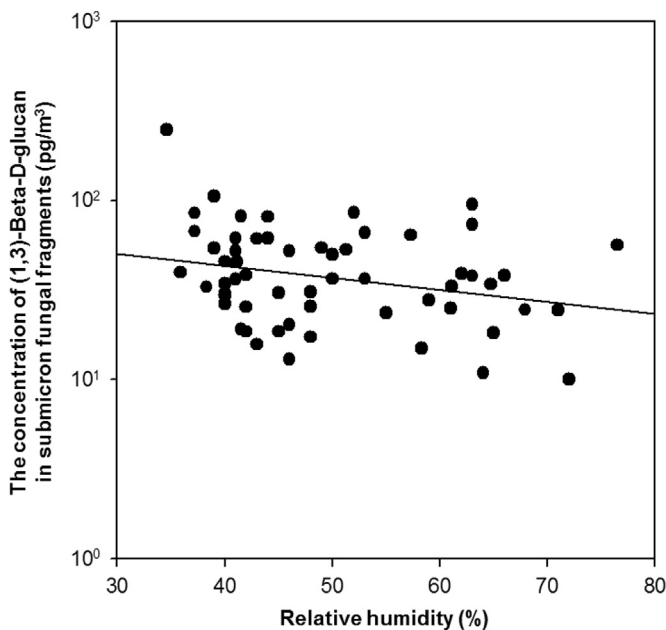
### 4. Discussion

Many epidemiological studies have reported that fine particulate matter (PM), especially PM<sub>2.5</sub>, as well as mold are strongly associated with the severity of asthma symptoms (Andersen et al., 2012; Dales et al., 2009; Iskandar et al., 2012; Mann et al., 2010; Mar et al., 2010; Qian et al., 2009; Wiwatanadate and Liwsrisakun, 2011). However, the contribution of fine fungal particles to human illness is poorly characterized since the quantification of these particles in damp buildings has previously been hindered by the lack of suitable size-selective sampling and analytical methods. Recently, exposure assessment of fine fungal particles has been performed in several studies as a new sampler capable of size-selective collection has been developed and analytical methods

**Table 2**  
Pearson correlation coefficients among the concentration of submicron fungal fragments, airborne mold, temperate, and relative humidity.

	Temperature	Relative humidity	Airborne mold	Submicron fungal fragments (FF)	Log-transformed airborne mold	Log-transformed FF
Temperature	1.000	−0.058 ( <i>P</i> =0.423)	−0.023 ( <i>P</i> =0.604)	−0.130 ( <i>P</i> =0.733)	−0.020 ( <i>P</i> =0.506)	−0.004 ( <i>P</i> =0.726)
Relative humidity	–	1.000	0.119 ( <i>P</i> =0.522)	− <b>0.257</b> ( <i>P</i> = <b>0.046</b> ) <sup>†</sup>	0.081 ( <i>P</i> =0.524)	− <b>0.270</b> ( <i>P</i> = <b>0.044</b> ) <sup>*</sup>
Airborne mold	–	–	1.000	−0.001 ( <i>P</i> =0.587)	<b>0.971</b> ( <i>P</i> < <b>0.0001</b> )	0.082 ( <i>P</i> =0.501)
Submicron fungal fragments (FF)	–	–	–	1.000	0.021 ( <i>P</i> =0.540)	<b>0.874</b> ( <i>P</i> < <b>0.0001</b> )
Log-transformed airborne mold	–	–	–	–	1.000	0.101 ( <i>P</i> =0.462)
Log-transformed FF	–	–	–	–	–	1.000

<sup>\*</sup> *P* values are mentioned in the parenthesis, and significant correlations are marked bold.



**Fig. 3.** The scatter plots between the concentrations of (1,3)- $\beta$ -D-glucan in submicron fungal fragments and relative humidity ( $ar^2=0.071$ ,  $P=0.004$ ).

using measurement of surrogate makers in fungal components such as (1,3)- $\beta$ -D-glucan have become commercially available (Reponen et al., 2007; Seo et al., 2008, 2009). Nonetheless, these studies investigated only the level of submicron fungal fragments in homes with water damage or that were affected by the Hurricanes Katarina and Rita, which make it difficult to predict the effect of these fine particles on health outcomes in more general settings. To our knowledge, our study is the first to measure and compare the levels of submicron fungal fragments in homes with asthmatic and non-asthmatic children. In particular, our study demonstrates that the indoor concentration of (1,3)- $\beta$ -D-glucan in submicron fungal fragments was significantly higher in homes with asthmatic children than in similar homes with non-asthmatic children. We believe that these findings could be utilized as background information for investigating the role of fine fungal particles on health outcomes of allergic diseases in the future since these particles are more common than airborne spores and may be one of the main sources of allergens as reported by a previous study (Green et al., 2006).

Very few studies evaluating the level of submicron fungal fragments in homes are available. One study reported that the GM concentrations of (1,3)- $\beta$ -D-glucan in submicron fungal fragments measured in homes with water damage and visible mold or that

were flooded by a hurricane were 59.6–192.7  $pg/m^3$  in summer and 338.0–520.5  $pg/m^3$  in winter, which indicated that the concentrations in winter are up to about 5.7 times higher than those in summer (Reponen et al., 2007). Considering that our sampling was performed between the fall and early winter in Korea, the GM concentration levels (26.7–50.9  $pg/m^3$ ) in our study were lower than those in the previous study. The difference may be due to the fact that the levels of submicron fungal fragments in the previous study were measured in homes affected with extreme water damage or flooding. This can be seen by comparing the concentrations of airborne mold. The GM concentrations (940.5–7182.8 CFU/ $m^3$ ) of airborne mold in winter in the previous study was up to 60 times higher than those (122.8 CFU/ $m^3$ ) in our study (although it should be noted that the concentration of airborne mold was not correlated with the level of submicron fungal fragments in either study). However, the concentration range of submicron fungal fragments in homes with an asthmatic child was 17.2–247.1  $pg/m^3$ , which is in the same order of magnitude as the previous work. This finding indicates that much exposure to submicron fungal fragments may occur to asthmatic children, and this is why a further study is necessary for investigating their health impact.

In the present study, the GM indoor concentration of submicron fungal fragments in homes with asthmatic children was about 2-fold higher than that in homes with non-asthmatic children or than that in outdoor. The 1.6-fold greater concentration of airborne mold (135.5 CFU/ $m^3$  in asthmatic group vs. 85 CFU/ $m^3$  in non-asthmatic group) may partly explain this difference, though there was no associations between the levels of airborne mold and fine fungal particles as mentioned earlier. This difference could be explained by differences in relative humidity. In homes with an asthmatic child, it is likely that relative humidity has been monitored for symptom alleviation in terms of the intervention for growth of biological factors such as house dust mites (HDM). The low relative humidity could contribute to easy aerosolization of submicron fungal fragments and lead to an increase in the levels of submicron fungal fragments in indoor air. This might be supported by our finding that the mean relative humidity in homes with asthmatic children ( $48.5 \pm 10.4\%$ ) was lower than that in homes with non-asthmatic children ( $51.2 \pm 11.1\%$ ) though the mean relative humidity in both groups was not significantly different. Regarding the comparison between indoor and outdoor concentrations, some plants may affect the outdoor concentrations of (1,3)- $\beta$ -D-glucan (Douwes, 2005). The lower concentrations of (1,3)- $\beta$ -D-glucan in outdoor might be expected since most of the homes in this study are located in old downtown with few parks and trees. Also, about 40% of asthmatic children lived in homes located on the ground floor, which may facilitate mold growth as indicated by a previous study (Roussel et al., 2008). Relative higher concentrations of airborne mold in homes with asthmatics may support this explanation as well.



The aerosolization of fungal spores has been shown to be affected by physical factors such as indoor air current, vibration, and relative humidity (Foarde et al., 1999; Górný et al., 2001; Górný et al., 2003; Madelin, 1994; Pasanen et al., 1991). Particularly, relative humidity was inversely proportional to the aerosolization of fungal spores (Foarde et al., 1999; Pasanen et al., 1991), which is consistent with our findings. The sources of submicron fungal fragments would be the fragments of spores or mycelium from the hyphal structure and it is estimated that low relative humidity facilitates the formation of fine particles (i.e., breaking the hyphal structures) by desiccating the surfaces of spores and mycelium. It is thus believed that low relative humidity has an association with the high concentration of submicron fungal fragments. Nonetheless, a further study is more necessary for exploring the process of aerosolization since this is dependent on the species and the types of surfaces on the walls or ceilings (Górný, 2004; Green et al., 2006).

The relative lower incidences of visible mold or water stain in homes with asthmatics were observed counter-intuitively in this study (Table 1). This may be attributed to their parents' frequent and active cleaning practices to avoid or lower the exposure to environmental risk factors (i.e., mold) (Yoon et al., 1999). Nonetheless, the level of submicron fungal fragments was higher in homes with asthmatics than that in homes with non-asthmatics in this study. This result may confirm that the presence of visible mold or water stain may not be appropriate for assessing exposure to mold, in particular examining the association between exposure to mold and health symptoms as a recent study indicated (Vesper, 2011). For this reason, additional sampling and analysis for fine fungal particles could be a good alternative for assessing exposure to mold in homes with allergic diseases.

Asthma is understood to be a chronic disease of the airways characterized by an inflammatory response (Institute of Medicine, 2000; NHLBI, 2007). It should be noted that both genetic and environmental factors such as environmental tobacco smoke (ETS), air pollution (i.e., particulate matters and ozone), allergens produced by cats, dogs, cockroaches, mold, and house dust mites, and their by-products (i.e., mycotoxin and endotoxin) or biological active components (i.e.,  $\beta$ -D-glucan) appear to play important roles in initiation and exaggeration of asthma symptoms (Institute of Medicine, 2000). Moreover, behavioral and housing characteristics (i.e., housing types, location, and housing quality) as well as socioeconomic status (SES) of household have been shown to be associated with development or exaggeration of asthma symptoms (Farfel et al., 2010; Heinrich, 2011; Hong et al., 1994; Northridge et al., 2010). Asthma is thus an illness attributed to a complex interaction of these environmental risk factors, not one or two risk factors only. For these reasons, one important limitation that needs to be acknowledged is that our study only examined fungal spores and fragments, and explored physical factors (i.e., relative humidity and temperature) with the level of airborne fungal fragments. Also, the types of mold, maturity, and seasonal factors as well as the difference of house structure could affect the concentration of (1,3)- $\beta$ -D-glucan in submicron fungal fragments, but these were not considered in this study. The association of severity of asthma symptoms with the level of submicron fungal fragments was not analyzed due to time difference between air sampling and previous results of lung function tests.

In conclusion, the concentrations of (1,3)- $\beta$ -D-glucan in submicron fungal fragments in homes with an asthmatic child sensitized to mold were significantly higher than the outdoor concentrations or those in homes with non-asthmatic child. Therefore, our findings indicate that the submicron fungal fragments would be another type of exposure to mold, and the exposure assessment for submicron fungal fragments with more standardized analytical methods (i.e., the molecular-based analysis

or surrogate analysis using fungal biomass) should be included when evaluating the effects of mold on asthma symptoms in the future. As low relative humidity in indoor air was found to increase the level of submicron fungal fragments, the very careful control of relative humidity would be necessary for prevention and alleviation of the severity of asthma sometimes for inhibiting the growth of microorganisms (i.e., mold and HDM) or decreasing the release of (ultra)fine fungal particles.

## Disclaimer

The findings and conclusions in this paper are those of the authors and do not necessarily represent the views of the Ministry of Environment, Republic of Korea (MOE) and the United States National Institute for Occupational Safety and Health (NIOSH). The mention of any company names or products does not imply an endorsement by MOE and NIOSH, nor does it imply that alternative products are unavailable, or unable to be substituted after appropriate evaluation.

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