

A dose-dependent relationship between the severity of visible mold growth and IgE levels of pre-school-aged resident children in Taiwan

Abstract To demonstrate a dose-dependent relationship between severity of indoor visible mold growth and serum total IgE levels of resident children. A total of 97 children (4–7 years old) identified from previously established birth-cohort, with information pertaining to indoor environmental conditions after child's birth, were successfully recruited while sera were concurrently collected for total IgE and specific IgE analysis during clinical visits. Severity of visible mold growth at homes was scaled into three levels accordingly. A statistically significant dose-dependent relationship was found between severity of indoor visible mold growth and total serum IgE levels. The trend sustains after the model was adjusted for resident child's age, gender, pet-keeping history, number of siblings, atopic history of parents, presence of incense burning, and environmental tobacco smoking (ETS) at home. Further analysis of specific IgE to commonly examined fungal allergens did not substantiate the correlation. Rather, resident child's specific IgE to mite allergens, although without statistical significance, seemed to better associate with the ranked severity of indoor mold growth in this study. An adjuvant role of fungal exposure to enhance sensitization in indoor environment is therefore suggested in Taiwanese population with high prevalence of building dampness.

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Practical Implications

The presence of indoor visible mold growth, potentially resulting in fungal exposure, was not associated directly with changing biomarker levels of allergic response in resident children, rather playing an adjuvant role to enhance sensitization. On the other hand, other allergens, such as mite allergen examined in this study, appeared to support a more plausible etiology for directly triggering the ultimate allergic symptoms and diseases of interest. Evidence as such may derive different priority-setting when designing preventive measures for managing indoor air quality.

Introduction

Environmental risks, especially bioaerosols exposure, have been attributable to the rising prevalence of childhood allergic diseases (Zeldin et al., 2006). Building dampness and indoor mold exposure are associated with increasing frequency of reporting allergic and respiratory symptoms or illnesses in extensive epidemiological studies across different geographic and ethnical backgrounds even using only questionnaires to document the symptoms frequency or disease prevalence (Bornehag et al., 2005; Lee et al., 2003; Park et al., 2004; Tham et al., 2007). Yet, very few have performed direct measurements, for examples, of

changes in lung function, airway reactivity (Hagmolen of Ten Have et al., 2007), or markers of inflammation to characterize objectively the health effects of interests (Hellgren et al., 2009; Hirvonen et al., 1999; Thorn and Rylander, 1998; Walinder et al., 2001). To date, no study has established any dose-dependent relationship between observed levels of indoor mold exposures and related changes in biomarker concentrations.

Scientific understandings remain controversial and inconclusive regarding the role of indoor fungal exposure to the development or exacerbation of allergic diseases for several reasons. One, although many fungi have shown their capacity in mediating Type I allergy (Breitenbach, 2008), sensitization rates to most

environmental fungi, compared to that to mite or other allergens, were relatively low, mostly below 20% in asthmatic or allergic subjects of various age groups (Ezeamuzie et al., 2000; Mari et al., 2003; Niedozytko et al., 2007; Nolles et al., 2001; Prasarnphanich and Sindhurat, 2005; Reijula et al., 2003). Meanwhile, a nonspecific inflammatory reaction, not a direct IgE-mediated allergy, was proposed to characterize the necessary biological mechanism initiated after exposure to indoor molds (Douwes et al., 2000a; Hirvonen et al., 1999; Purokivi et al., 2001; Rylander et al., 1998). Nevertheless, repeated associations found in serial questionnaire surveys between the dampness of buildings and reporting allergic symptoms and/or diseases of residents, often with high significance, could not be accounted simply by nonspecific inflammatory effects alone (Bornehag et al., 2005; Tham et al., 2007). As no satisfactory underlying mechanism is yet presented, it is speculated that some readily available contaminants, such as mite allergens often rich in most damp or moldy buildings, or other yet-to-be identified agents, might have had a critical role in inducing the final allergic response as observed.

Taiwan, with year-round high temperatures and humidity, sustains an ideal environment for indoor mold growth. For culturable fungi in general residence ($n = 35$), the highest concentration was 20551.7 CFU/m³ measured in winter, whereas the lowest being 6798.1 CFU/m³ in summer (Su et al., 2001a). The high levels of indoor fungi and other biological pollutants are concurrently available with an unique seasonal pattern different from assessments of western hemisphere (Horner et al., 2004; Su et al., 2001a,b; Toivola et al., 2004). This study was therefore aimed at demonstrating a correlation between severity of mold exposure and concentrations of serum IgE, an indicator often considered to suggest the risk for developing allergic illnesses. The analysis of specific antibody was examined to further verify whether the changing levels of allergic response biomarker was contributed simply by exposure to indoor fungi, or requiring the co-presence of other allergens.

Material and methods

Study subjects and follow-up

Pregnant women were interviewed while visiting Gynaecology and Obstetrics Clinics of participating hospitals in southern Taiwan. Upon agreement, a total of 957 to-be-born children were registered into the cohort between 1998 and 2000. Information on the housing characteristics and other hereditary attributes of both parents was also documented. At the time this study took place, children had already grown to be 4–7-years-old. Mother of each study child was individually interviewed by telephone for the indoor environ-

mental conditions and child's health status at the time of inquiry. After all these passing years, a total of 425 (44.4%) was successfully retrieved to participate in the telephone interview. The rest was not available for reasons including declining the invitation directly by the subject mother or her family, telephone number no longer valid, no response after three callings at different days, among others. A subsample of 114 children (26.8%), by the consent of their parents, agreed to make return visits to the clinics for health consultation and serum analysis. Fourteen of them refused to provide serum sample and three children did not complete follow-up questionnaire information, resulting a total of 97 study subjects left for final data analysis. Difference analysis was conducted between the study group of 114 and the starting group of 425. A number of variables were compared, including the gender, number of sibling, age of mothers at child birth, as well as percentage of mold growth at walls. No statistical difference was found for all the above. The representativeness of the study group in reference to the original cohort is presumed. This study was approved by the Human Experiment and Ethic Committee of National Cheng Kung University Hospital.

Total IgE concentration and Phadiatop® assay

Children's sera were collected for the analysis of total IgE, ImmunoCAP Phadiatop® and fx5 assay (Pharmacia Diagnostics AB, Uppsala, Sweden; detection limit 0.35 kU/l). The Phadiatop® assay was carried out according to the manufacturer's instructions using the UniCAP automated system and included the following allergens: Mites (*D. pteronyssinus* and *D. farlane*), pets (cat and dog), mixed molds (*Penicillium*, *Cladosporium*, *Aspergillus*, and *Alternaria*), mixed grasses (*Parietaria*, *Lolium*, *Phleum*, and *Cynodon*), *Artemisia* and mixed trees (*Acer*, *Betula*, *Ulmus*, *Quercus*, *Olea*, *Salix*, *Pinus*, *Eucalyptus*, *Acacia*, and *Malaleuca*). The fx5 are commercially available products consisting food allergen mixture of milk, egg white, fish, peanut, wheat, and soybean.

Specific IgE to mold and mite

The *allergic children group*, a total of 57, defined as those who received either Phadiatop® score or fx5 above 0.35 kU/l, was selected for specific IgE analysis afterward. Previous research, including ours, has not only identified that *Cladosporium*, *Penicillium*, and *Aspergillus* are among fungal genera prevalent in the general environments, but also often been implicated with allergic and respiratory outcomes (Rao et al., 2007; Su et al., 2001a; Toivola et al., 2004). In particular, a pilot study conducted indoor investigation into 20 residents of the current population had reported the most frequently present fungal genera included

Cladosporium (78.51%), *Penicillium/Aspergillus* (7.83%), Ascospores (3.74%), and Periconia (3.44%) After adjusting for the episode of fever, child's age, gender, and indoor CO₂ levels, significant associations were found between child's reporting frequency of sneeze and exposure of both outdoor and indoor airborne *Penicillium/Aspergillus* (Su et al., 2007). Therefore, *Cladosporium*, *Penicillium*, and *Aspergillus* became target allergic genera for specific IgE for analysis. The available commercial products, mx1 (*Penicillium chrysogenum* (*P. notatum*), *Cladosporium herbarum*, *Aspergillus fumigatus*, *Alternaria alternata*) and mx4 (*A. fumigatus*, *A. niger*, *A. terreus*, *A. flavu*) were the final testing ingredients. In addition, specific IgE (sIgE) for *Der p1* (*Dermatophagoides pteronyssinus*) was also included for analysis to compare the relative contribution of exposure to indoor visible molds and residential mites to the antibody loadings of study children.

Severity of visible mold at home

Housing conditions, including levels of dampness and severity of mold growth, were provided by mothers or caregivers of study children through telephone interview. When a positive response was given concerning 'the presence of visible mold on the wall', the question of 'how many walls with mold did they ever see?' would be further approached. In this study, severity of visible mold at homes was categorized into three levels: *No visible mold on the wall*, *visible mold on 1~2 walls*, and *visible mold on 3 walls or more*.

Data analysis

Total IgE levels were log transformed to achieve normal distribution. Comparison of total IgE levels between different characteristics was analyzed by *t*-test or one-way ANOVA. Trend test was applied using multiple linear regression for continuous data and chi-square for trend test (ordinary by ordinary) for ordinal data to examine the dose-response correlation between total serum IgE or specific IgE levels and severity of indoor visible molds.

Results

Table 1 shows the demographic characteristics and health status of 97 children. The average total serum IgE level was 256.5 kU/l (1, 3354 kU/l). Disease prevalence based on parental reporting of doctor diagnosis was different from that of calculation after clinical examination by physicians on-site. Prevalence of allergic rhinitis was found particularly to have been underestimated by parents' questionnaire response.

The associations between levels of indoor visible mold growth and the allergic status of study children,

Table 1 Characteristics of 97 study children

Characteristic variable	
Age, mean $\pm$ s.d.	5.3 $\pm$ 0.6 years
BMI, mean $\pm$ s.d.	15.8 $\pm$ 2.0
Total IgE level, mean $\pm$ s.d.	256.5 $\pm$ 483.2 kU/l
Male, % (n)	53.6 (52)
Maternal history of allergy, % (n)	37.5 (36)
Paternal history of allergy, % (n)	34.7 (33)
Sibling, % (n)	72.2 (70)
Birth order, % (n)	
First	41.2 (40)
Second	43.3 (42)
Third or more	15.5 (15)
Phadiatop [®] $\geq$ 0.35 kU/l, % (n)	49.5 (49)
fx5 (milk, egg white, fish, peanut, wheat, and soybean) $\geq$ 0.35 kU/l, % (n)	37.1 (36)
Self-reported physician-diagnosed disease ^a , % (n)	
Asthma	19.8 (19)
Eczema	28.9 (28)
Allergic rhinitis	26.0 (25)
Allergic conjunctivitis	24.7 (24)
Physician diagnosis during clinical visit ^b , % (n)	
Asthma	16.8 (16)
Eczema	10.6 (10)
Allergic rhinitis	56.7 (55)
Allergic conjunctivitis	21.1 (20)

^aThe reported symptom in questionnaire ascertained by telephone visit.

^bThe symptom was consulted by pediatrician during clinical visit.

categorized by total IgE levels, Phadiatop[®] score, fx5 $\geq$ 0.35 kU/l, and disease prevalence, were examined in Table 2. The median values of IgE concentration in three groups were 50.9, 113.0, and 251.5 kU/l, respectively. A statistically significant dose-dependent relationship was established between the severity of indoor visible mold and total serum IgE levels of resident children ($\beta = 0.28$, $P = 0.007$) by univariate linear regression. The correlation remained significant ($\beta = 0.26$, $P = 0.007$) after adjusting for resident child's age, gender, pet-keeping, number of siblings, atopic history of parents, presence of incense burning, and environmental tobacco smoking (ETS) at home. Yet, as shown in Figure 1, such a dose-dependent trend with significance was found only for those children with allergy with positive response to the Phadiatop[®] analysis or fx5 $\geq$ 0.35 kU/l.

Phadiatop[®] is often adopted as a convenient measure to determine the atopic status of subjects. This study identified a significant trend between the severity levels of visible mold growth and the percentages of allergic children in the group (P for trend = 0.017, Table 2). In addition, children having a fx5 level $\geq$ 0.35 kU/l are considered to present allergy to any of the following food item, including milk, egg white, fish, peanut, wheat, or soybean. We conducted both panels of analysis with the intent to retrieve as much atopic information of the study subject as possible as it can be critical modifiers before concluding how the fungal exposure may be associated with the measured

Table 2 Correlation between reported mold severity and on children' total IgE concentrations, Phadiatop®, f × 5 analysis, and allergic diseases prevalence

Indicators of children' allergic status	Severity of visible mold, % (n)			P-value	P-value for trend ^{a,b}
	No mold on the wall, 46.9 (45)	On 1~2 walls, 32.3 (31)	On ≥ 3 walls, 20.8 (20)		
Total IgE; median (25th–75th percentile), kU/l	50.9 (15.0–128.5)	113.0 (64.7–350.0)	251.5 (13.2–606.3)	0.005	0.007^a
Phadiatop® ≥ 0.35 kU/l, % (n)	35.6 (16)	64.5 (20)	60.0 (12)	0.028	0.017
fx5 ≥ 0.35 kU/l, % (n)	28.9 (13)	41.9 (13)	50.0 (10)	0.22	0.08
Prevalence of self-reported physician-diagnosed disease, % (n)					
Asthma	20.0 (9)	19.4 (6)	20.0 (4)	>0.95	>0.95
Eczema	24.4 (11)	25.8 (8)	40.0 (8)	0.41	0.28
Allergic rhinitis	22.2 (10)	25.8 (8)	35.0 (7)	0.56	0.32
Allergic conjunctivitis	26.7 (12)	19.4 (6)	25.0 (5)	0.76	0.71
Prevalence of physician diagnosis during clinical visit, % (n)					
Asthma	18.2 (8)	10.0 (3)	25.0 (5)	0.37	0.82
Eczema	7.0 (3)	13.3 (4)	15.0 (3)	0.54	0.28
Allergic rhinitis	51.1 (23)	61.3 (19)	60.0 (12)	0.63	0.40
Allergic conjunctivitis	14.0 (6)	19.4 (6)	40.0 (8)	0.06	0.03

^aP-value for trend calculated by multiple linear regression adjusted for child's age, gender, siblings, atopic history of parents, presence of incense burning, having pet at home, and having environmental tobacco smoking at home among continuous data. Bold values presented as significant results ($P < 0.05$).

^bP-value for trend calculated by chi-square for trend (ordinal by ordinal) test among nominal data.

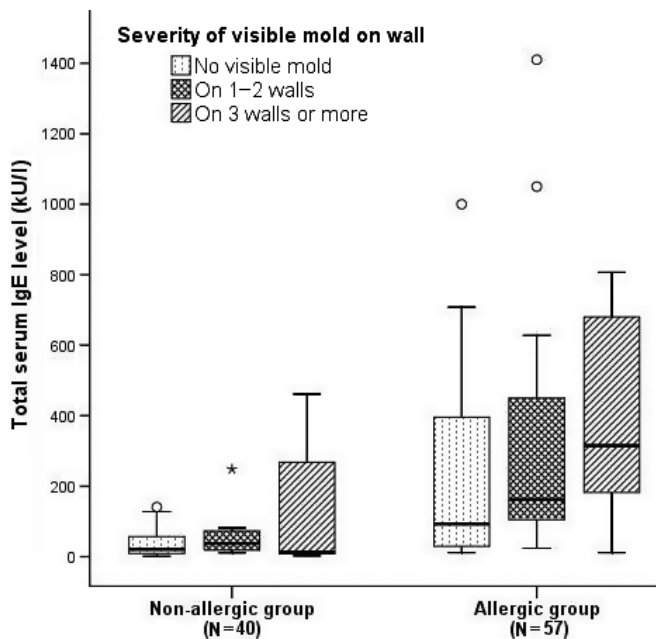


Fig. 1 Relationships between ranked severity of mold growth and total IgE on allergic (P for trend = 0.02) and non-allergic (P for trend = 0.27) children

immunological responses. There was also an increasing trend of the prevalence of allergic conjunctivitis with the increasing severity of mold exposure at home.

As the total IgE of study children was found significantly correlated with levels of indoor mold growth, analysis of specific IgE (sIgE) to fungi and other allergens was further carried out. Table 3 lists the positive sensitization rates to mite or pooled mold allergens. There was no trend of significant between the categorical severity of visible mold growth and

percentage of sensitization rate to allergens of mite or environmental fungi analyzed. However, if the numeric values of specific IgE were used for analysis (Table 4), an increasing trend for sIgE to mite allergen, although without statistical significance, was found with the higher severity of reported visible mold growth (median level of sIgE: 2.98, 4.52, 7.63 kU/l). The spearman correlation between levels of total IgE and specific IgE to mite was 0.33, $P = 0.01$.

Discussion

A significant dose-dependent relationship is found between severity of visible mold growth at homes and total IgE levels of study children, one indicator often considered to suggest the risk for developing allergic illnesses. Yet, analysis of specific IgE to fungal allergens does not further substantiate the correlation. Rather, while levels of specific IgE to *Der p1* allergens are not significantly associated with corresponding total IgE, they appear to better correlate with the severity of indoor mold growth as ranked in this study. It was therefore speculated that the health outcomes implicated by presence of indoor molds in previous researches might have been much attributable to the unmeasured concurrent exposure to other indoor biological pollutants, including residential mites, rather than the direct effects resulting from exposure to indoor fungi, as often suggested due mostly to the visible presence of mold growth. It is highly plausible that fungal exposure posed mainly an adjuvant effect in this scenario.

A serial questionnaire surveys have repeatedly suggested associations between the dampness of buildings and reporting allergic symptoms and/or diseases of residents, often with high significance (Bornehag et al.,

Table 3 The allergic prevalence to specific allergen by ranked severity of visible mold growth

Positive rate of specific allergen (Positive if sIgE $\geq$ 0.1 kU/l)	Severity of visible mold			P-value	P-value for trend
	No mold on the wall (n = 22)	On 1~2 walls (n = 23)	On $\geq$ 3 walls (n = 12)		
<i>Dermatophagoides pteronyssinus</i> , % (n)	72.7 (16)	91.3 (21)	66.7 (8)	0.16	0.92
mx1 (<i>Penicillium chrysogenum</i> (P. notatum), <i>Cladosporium herbarum</i> , <i>Aspergillus fumigatus</i> , <i>Alternaria alternata</i>), % (n)	36.4 (8)	30.4 (7)	50 (6)	0.52	0.60
mx4 (<i>A. fumigatus</i> , <i>A. niger</i> , <i>A. terreus</i> , <i>A. flavus</i>), % (n)	4.5 (1)	8.7 (2)	0 (0)	0.54	0.78

Table 4 Association between ranked severity of mold growth and numeric levels of specific IgE

Level of specific IgE antibodies (kU/l), median (25th–75th percentile)	Severity of visible mold			P-value	P-value for trend
	No mold on the wall (n = 22)	On 1~2 walls (n = 23)	On $\geq$ 3 walls (n = 12)		
<i>Dermatophagoides pteronyssinus</i>	2.98 (0.08–9.84)	4.52 (0.31–55.9)	7.63 (0.04 to >100)	0.39	0.34
m $\times$ 1 (<i>Penicillium chrysogenum</i> (P. notatum), <i>Cladosporium herbarum</i> , <i>Aspergillus fumigatus</i> , <i>Alternaria alternata</i>)	0.06 (0.02–0.38)	0.04 (0.02–0.12)	0.08 (0.03–0.35)	0.41	0.84
m $\times$ 4 (<i>A. fumigatus</i> , <i>A. niger</i> , <i>A. terreus</i> , <i>A. flavus</i>)	0 (0–0.01)	0 (0–0.03)	0.01 (0–0.06)	0.71	0.22

2005; Tham et al., 2007). In this study, total IgE of study children increased with the ranked severity of indoor visible mold growth, an observation may be adopted to imply that our study subjects may take on a type I hypersensitivity response at the presence of indoor visible molds. Moreover, a quantitative dose–response relationship between the exposure to viable, airborne fungi and the total IgE was found among children with asthma ($r = 0.44$ and $P = 0.02$, $n = 28$) of the same geographic region in our previous study (Su et al., 2005). Investigation from other countries has also reported a similar association between exposure to molds in a water-damaged school and an increase in specific IgE sensitization of children using the same Phadiatop® test (Savilahti et al., 2001) as applied in our current study. Nevertheless, only limited researches have offered direct evidence with quantitative levels or changing compositions of the indoor fungi to support the health implications of exposure to damp buildings or indoor visible molds; of which even fewer have conducted concurrent assessment of multi-microbial agents. Douwes et al. (2000b) measured the house dust for the levels of *Der p1*, endotoxin and (1 $\rightarrow$ 3)- β -D-glucan, an water-insoluble structural cell wall component in most fungi, all from the same sample. High levels of glucan was found to affect significantly the peak expiratory flow variability in atopic children only, regardless of asthmatic status, after adjusting for *Der p1* level, presence of pet and carpet. It was interpreted that the fungal exposure would not only be associated with reporting respiratory symptoms related to inflammation, but also linked to inducing allergic symptoms associated with IgE antibody response.

Unlike serum total IgE, children's specific IgE to various fungal genera prevalent in this geographic region was not necessarily correlated with ranked severity of visible mold growth in this study. One might wonder whether the environmental fungi had ever resulted in any meaningful exposure. Using a randomly selected subset of 20 households from the current study population, higher median concentration for homes with visible mold on wall (15014.5 CFU/m³, $n = 8$), compared to that without (2550.0 CFU/m³, $n = 10$), was found in our previous investigation. While the ranked severity of mold growth appears to be a useful surrogate indicator for the actual fungal exposure levels, sIgE to measured fungi is, on the other hand, not identified. We therefore speculated that fungus might not be the major sensitizer contributing to the increasing levels of total IgE. The role of other biological agents, like house dust-mites, was also taking into consideration as they are also highly associated with the presence of 'dampness' or 'visible mold on wall' in indoor environment, along with higher levels of fungi and bacteria (Pennanen et al., 2007). In this study, the specific IgE to *Der p1* presented a dose-dependent increase with observed severity of visible mold growth, although without statistical significance due possibly to small sample size. Nevertheless, the finding argues that other more effective sensitizers, such as mite allergen, may be present in the study environment and warrant further investigations.

If indoor fungi were not the direct triggers to allergic response measured in this study, what was the exact role of fungal exposure to allergic outcomes of interest?

Several animal studies, exposing mouse simultaneously to an experimental allergen (ovalbumin; OVA) and fungus, had resulted in similar observation where sIgE to OVA would increase significantly with the copresence of selected fungi, suggesting that exposure to indoor fungi might exert an adjuvant effect. The IgE and IgG₁ levels both indicated a T_H2 response in mice. Ormstad et al. (2000) used fungal indicator of β -1,3-D-glucan, major structural component of the cell wall from fungus, and exposed mice to OVA and glucan simultaneously. Both OVA-sIgE and IgG₁ were higher than those with exposure to OVA or β -1,3-D-glucan independently and neither had any changes in IgG₂ associated with T_H1 response. Instanes et al. (2004) tested β -1,3-D-glucan extracts collected from *Cladosporium herbarum* and *Penicillium chrysogenum*. Levels of OVA-specific IgE and IgG₁ increased after exposure to combined allergen of OVA and extracted fungi, compared to using OVA alone. Kobayashi and his colleagues, using OVA and a common allergen of short ragweed pollen, demonstrated a similar pattern of potent T_H2-like adjuvant effects in the airways through antibodies and cytokine measurement with *Alternaria* exposure (Kobayashi et al., 2009). Consistent findings from these well-characterized animal studies concluded that the added presence of environmental fungi would exacerbate the response to T_H2 cell pathway and resulted in higher allergen-specific antibodies, a phenomenon called 'adjuvant effect'. Specifically, that environmental fungi do not seem to pose an allergenic effect itself whereas with great possibility to enhance sensitization. Such findings also offered justifications as to why even with the positive associations of dampness/visible mold and health outcome being found repeatedly, the sensitization rate to analyzed fungal genera was usually low in most prevalence studies (Bornehag et al., 2005; Lee et al., 2003; Park et al., 2004; Tham et al., 2007).

How are fungi able to exert an 'adjuvant effect'? A series of studies have demonstrated with cytokine

measurement that people staying in damp environment are prone to provoke respiratory inflammatory response (Hellgren et al., 2009; Hirvonen et al., 1999; Purokivi et al., 2001; Walinder et al., 2001), and proteases in fungal extracts are capable of affecting airway epithelium where inflammatory cytokines are generated (Kauffman et al., 2000). As pro-inflammatory cytokine, IL-6, would suppress IL-12-mediated T-cell polarization and direct T_H2 differentiation of naïve T cells (Rincón et al., 1997), fungus exposure of *Alternaria* was found to potently induce the expression of IL-6 and OX40L by dendritic cells (Kobayashi et al., 2009). More comprehensive underlying mechanisms remained to be studied in greater details.

In summary, this study with environmental and biomarker assessment identified a significant dose-dependent relationship between severity of visible mold growth at home and children's total IgE levels. An adjuvant role of fungal exposure to enhance sensitization in indoor environment was also suggested in Taiwanese population with high prevalence of building dampness. To the best of our knowledge, this field study may be the first quantitative, biological evidence to suggest that health implications, especially using changing biomarker levels as surrogate indicators, might not be attributable entirely to fungal exposure using visible mold growth as surrogate, whereas other allergen exposure might support a more direct etiology. The specific underlying etiology of fungal and allergen exposures to result in the observed health outcomes warrants future investigations and interaction was need for future study.

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