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The effect of home characteristics on dust antigen concentrations and loads in homes

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Abstract

On-site home visits, consisting of a home inspection, dust sampling, and questionnaires were conducted in 777 homes belonging to an ongoing birth cohort study in Cincinnati, Ohio. Various home characteristics were investigated, and antigen levels (concentrations [μg/g] and loadings [μg/m^2]; IU for cockroach allergen) in floor dust samples collected in child's primary activity room were analyzed by ELISA. Monoclonal antibodies were used for the analysis of cat, house dust mite, and cockroach allergens, and polyclonal antibodies for *Alternaria* and dog antigens. The relationship between the antigen levels and home characteristics was investigated through a generalized multiple regression model.

More than half of the homes experienced mold/water damage. Cats and dogs were present in 19.7% and 31.1% of homes, respectively. More than 90% of homes had either carpet or area rug covering their floors. Among 777 homes, 87–92% of homes had measurable amount of *Alternaria*, cat, and dog allergen/antigen in house dust, whereas only 38% and 14% of homes had measurable levels of house dust mite and cockroach, respectively.

Alternaria antigen level in house dust was not associated with visual mold/water damage, which was suspected to be one of the sources for this antigen in homes. Instead, the antigen level was high in samples taken in fall and in homes having dogs implicating that *Alternaria* antigen appears to be transported from outdoors to indoors. A high level was also measured in homes using a dehumidifier (these homes have experienced excessive humidity) and in-home venting of clothes dryer, which might be associated with microclimate affecting mold growth and spore release. The allergen/antigen level (both concentration and loading) of cat, dog and cockroach was significantly associated with the number of cats and dogs, or the appearance of cockroaches, respectively. High level of house dust mite allergen was measured in bedrooms and in homes using dehumidifier and no central forced air heating system. Having indoor plants was shown to reduce allergen levels. Carpeted floor was found to hold larger amount of antigens than non-carpeted floor.

Antigen loading demonstrated more consistent and larger numbers of associations with home characteristics compared to antigen concentration. This study encompassed a wide range of home characteristics and various antigen types. Our findings provide information on home characteristics that can be used for allergen avoidance and in planning future exposure assessment studies.

Keywords

Alternaria; Cat; Dog; House dust mite; Cockroach; Allergen; Antigen; Home characteristics

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1. Introduction

The prevalence of atopic disorders has increased in recent decades (Åberg, 1989; Ninan and Russell, 1992; Peat et al., 1994; Akinbami and Schoendorf, 2002). Exposure to indoor aeroallergens (cat, dog, house dust mite, cockroach, mold) is of particular concern because about 75–80% of children with asthma have significant allergies, which can trigger asthma, and thus have considerable medical and economic impact (Platts-Mills and Carter, 1997; Ward et al., 1998; American Lung Association, 2006). There is evidence that animal, cockroach, and house dust mite allergen exposure occurring in early age can influence the future development of atopic conditions such as asthma and atopic dermatitis (Huang et al., 2001; Salam et al., 2004). Various studies have evaluated levels of indoor aeroallergens in order to investigate the development of atopic diseases in children (Squillace et al., 1997; Remes et al., 2001; Zock et al., 2002; Belanger et al., 2003; Salam et al., 2004; Gruchalla et al., 2005; Brussee et al., 2005). Specific levels of indoor allergens have been found to be associated with various home characteristics (Chew et al., 1998; Mihrshahi et al., 2002; Matheson et al., 2003; Foarde and Berry, 2004; Arbes et al., 2004; Gruchalla et al., 2005). Certain home characteristics such as carpeted floor and pet ownership have been included in many studies with different cohorts and were confirmed to influence indoor allergen levels. On the other hand, there are some aspects of indoor environment, which have not been extensively studied and would need reinvestigation with other types of co-existing home characteristics.

In the present study, we incorporated various home characteristics from previous studies in an effort to perform a comprehensive evaluation of indoor environment. Five different indoor antigens were analyzed to assess child's exposure, and those levels expressed as concentration and loading units were investigated in association with home characteristics.

2. Materials and methods

2.1. Studied homes

We evaluated 777 dwellings of infants participating in Cincinnati Childhood Asthma and Air Pollution Study (CCAAPS), a birth cohort study. Infants born in Cincinnati and Northern Kentucky between 2001 and 2003 were recruited using birth certificate data (Ryan et al., 2005). Eligibility for the study required that at least one parent was atopic, which was determined by positive skin prick test (SPT) response (wheel $\geq$ 3 mm) to at least one of 15 common aeroallergens.

2.2. Investigation of home characteristics

A checklist was developed to obtain information about home characteristics. Existing questionnaires and home inspection protocols, which were used in previous studies (Lebowitz et al., 1989; Aamodt et al., 1999; Toivola et al., 2002; Belanger et al., 2003) and those published by professional organizations or governmental agencies (U.S. EPA, 1994, 2001; NIOSH, 1999; ACGIH, 1999) were utilized in the development of the checklist. Specific home characteristics found to be associated with indoor contaminants in other studies were also included in the checklist (Kozak et al., 1979; Dornelas de Andrade et al., 1995; Almqvist et al., 1999). The checklist consists of two parts: a questionnaire with 36 questions administered to a parent in an interview during the home visit, and an inspection list to evaluate housing conditions with six home characteristic factors in primary activity room, bedroom, and basement and two factors in other rooms.

On-site home visits were performed by trained two-person teams in 777 homes of infants with mean age of 8 months. The questionnaire was administered to a parent regarding surrounding

environment, building characteristics, infants' activity patterns, pet ownership, and housekeeping. The status of the building was assessed by a visual observation. Each room including the basement and attic was inspected for signs of visible mold or water damage. Location of damage, changes in the color and integrity of the surface material, and the size of damaged surface were recorded. Tape samples were taken from mold-contaminated surfaces to identify fungal genera. The extent of mold and water damage in homes was categorized into three classes based on the questionnaire and observation data as described by Cho et al. (in press). Class 0 homes did not have any mold/water damage history or indication, Class 1 homes had either history or minor indication of mold/water damage, and Class 2 homes had at least 0.2 m^2 of visible mold on building surface. Additionally, temperature and relative humidity were measured and the presence of moldy odor was recorded in the infants' bedroom, basement, and the room where the child spent most of his or her time when awake, referred to as the child's primary activity room (PAR). The number of indoor plants and stuffed toys in the PAR and the infants' bedroom were also counted.

2.3. Exposure assessment of indoor antigens

The families were requested not to clean the floor for at least one day before the home visit. At the visit, a parent was asked to identify the PAR. Dust samples were collected from flooring materials in the PAR using a vacuum cleaner (Filter Queen Majestic®; HMI Industries Inc., Seven Hills, Ohio) at a flow rate of 800 L/min. A custom-made cone-shape HEPA filter trap (Midwest Filtration, Cincinnati, OH) was attached to a nozzle of the vacuum cleaner to collect the dust sample. This equipment was chosen based on a pilot study. In the pilot study, flow rates, pressure changes and collection efficiencies were tested using different types of filter traps. The HEPA filter trap showed a collection efficiency of over 95% for particles larger than 0.3 μm. The custom-made cone-shape HEPA filter trap (CCAAPS filter trap) increased the vacuum flow rate by 10% compared to a commercially available rectangular HEPA filter trap. The selected vacuum cleaner was able to maintain a high flow rate with only a 3% pressure drop when it was used with the CCAAPS filter trap.

For floor covered with carpet or area rug (referred to as carpeted floor), a dust sample was collected from an area of 2 m^2 at a vacuuming rate of 2 min/m^2 (1 min horizontally, 1 min vertically). Then, in order to increase the amount of fine dust, which is a fraction used for the antigen analysis, another dust sample was collected from the same area with a new filter trap. The first sample typically had human and animal hair and other coarse particle whereas the second sample had more fine dust. Fine dust from these two consecutive samples was combined for the analysis. For non-carpeted floor (hard wood, linoleum, tile, or sheet floor), only one sample was collected from the entire room at a rate of 1 min/m^2. The reason for using a different sampling protocol for non-carpeted floor is that dust can be vacuumed from non-carpeted surfaces faster than from carpet (Ewers et al., 1994; Reponen et al., 2002). Furthermore, the amount of fine dust from a duplicated sample from a non-carpeted floor was negligible. Information on the size of the sampled area and floor material was recorded. The exact location of the sampled area was also recorded so that future repeat samples could be collected from the same location. The sample was taken back to the laboratory in a cooler bag with ice. The dust sample was sieved (355 μm sieve), and the fine dust was divided into sub-samples and stored at −20 °C before analysis.

Antigens were extracted from the sieved fine dust (50 mg) into 1 mL of PBS-T (0.05% Tween 20 in phosphate buffered saline, pH 7.4) by mixing overnight at 4 °C using a shaker and centrifuged the next day. The supernatant was analyzed using a commercial antibody-based enzyme-linked immunosorbent assay (ELISA) (Indoor Biotechnologies, Inc., Charlottesville, VA). Cat (Fel d 1), house dust mite (Der f 1), and Cockroach (Bla g 1) allergens were analyzed by capture assay using monoclonal antibodies. Dog and *Alternaria* antigens were analyzed

with an in house-developed alkaline phosphate based inhibition ELISA assay using commercially available polyclonal antibodies (Greer Laboratories, Inc., Lenoir, NC).

Results were expressed as ng allergens/antigens (or IU for cockroach) per mL extract, and converted to μg allergens/antigens per g of sieved dust (concentration, μg/g) and μg allergens/antigens per m^2 of sampling area (loading, $\mu g/m^2$). Loading was obtained by multiplying the concentration with the weight of collected dust and dividing with the size of sampling area. The minimum value of detectable concentration was determined from each run of allergen analysis and varied as follows: 244–1000 ng/mL for *Alternaria*; 1–12.5 ng/mL for cat; 12–391 ng/mL for dog; 5–78 ng/mL for house dust mite; and 0.02–0.16 IU/mL for cockroach.

2.4. Statistical methods for investigation of the relationship between home characteristics and antigen levels

Multiple regression analyses were separately performed with antigen levels in concentration and loading as the dependent variables and home characteristics as the independent variables. There were large numbers of samples (%) of which antigen levels were less than the limit of detection (LOD). Distributions of detectable concentrations were right skewed. The geometric mean was calculated to estimate the centers of allergen distributions, after LOD values were divided by two. When the number of LOD values is large it is not possible to apply a monotonic transformation to the data to achieve normality and to analyze the data using ordinary or weighted least squares regression. A common methodology was sought that could be applied to the analysis of each antigen so that results could be interpreted in a similar manner. The levels of each antigen were grouped into mutually exclusive categories. Integer values equal to 0, 1, 2, 3, etc. were assigned to the antigen levels in the different categories. Category values were assigned ordinally according to the range of antigen levels included. Category frequencies were analyzed as multinomial outcomes using a Poisson log-linear model. The numbers in each category were determined by the clustering and separation of the data. Cumulative distributions were approximately at the following percentiles of the original data: 50%, 75%, 90%, 95% and 99%. For each antigen, LOD values were placed into the first category. If the percent of LOD values was greater than 50%, then the first category was larger than 50% and the numbers in the remaining categories were adjusted accordingly; the number in sequential categories decreased by about 50%.

As shown in Table 1, home characteristic data were divided into three groups with respect to their influence on antigen concentration: direct source/factor or carrier of antigens (group 1); indirect source or control factors of antigens (group 2); and general housing characteristics (group 3). Mold/water damage class was included in group 1 representing a source of indoor mold.

Preliminary correlation analyses were conducted for individual home characteristics in order to decrease the number of predictors for the final model. Those variables, which were associated with allergen level at the 15% significance level, were initially included in home characteristic group-specific multiple regressions. This level was chosen to allow for effect modification of associations with allergen levels due to correlations with other variables in group-specific multiple regressions.

In group-specific multiple regression models, a backwards stepwise elimination procedure and independent reviews of beta weights and *R* values at each stage were conducted to remove or maintain variables in the model, and it reduced the possibility of making a Type I error. Interactions that were *a priori* judged to be possibly related to antigen level were evaluated in the regression model. A 5% significance level was used to judge the significance of individual home characteristics and 15% for interaction effects. The 15% significance level for judging interaction was used to increase the power to detect interactions, since these can modify results

of main affects. At the final stage, variables that remained from each group were combined and final models were determined based on these significance levels. Analyses were carried out using the SAS procedures PROC GENMOD.

2.5. Reliability of home characteristics data

On-site home visits were repeated in 37 randomly selected homes out of the initial sample of 777 homes within 2 months of the initial visit. Information collected by questionnaire and home inspection at initial and repeat home visits were compared to evaluate the reliability of home characteristics data. Prior to analysis, several individual home characteristic factors, which described a certain type of home characteristic, were combined. For example, air circulation methods for cooling such as use of a fan and opening windows were combined and compared with methods that include air filtration. Forty-two variables were analyzed, of which five were continuous or ordinally scaled data, and 37 were dichotomous or nominally scaled categorical data. Intervals of agreement (or disagreement) were defined for continuous and ordinal items. For example, the evaluation of 'year in which home was built' was carried out after the difference between initial and repeat values was calculated for each home. Differences were coded as following: 0: $\leq \pm 5$ years, 1: $\geq \pm 5$ difference. Differences were analyzed using a sign test (binominal test of equality of proportions of agreement and disagreement). Agreement between initial and repeat values of dichotomous variables was analyzed using McNemar test, an adaptation of the chi-square formula to measure the change in repeated outcomes. Agreement for multilevel variables was assessed by calculating weighted Kappa statistics. Significance was assessed at an *a priori* 5% level for each characteristic. Analyses were carried out using SAS procedure PROC FREQ.

2.6. Variability of home allergen levels

Variability in allergen levels was investigated for 30 pairs of dust samples. Each sample in a pair was taken from the same home with the same PAR during the initial and repeat walkthrough, respectively. For each allergen, coefficient of variance (CV) was calculated for each pair and averaged over all pairs.

3. Results

On average, the infants spent 92% of their weekly time at home in either the living room (56%) or family room (36%) as their PAR. For children who did not spend all their time at home, the average percentage of their weekly time spent outside the home was 18%.

Table 1 presents the distributions of all three groups of home characteristics obtained through the questionnaire and visual observation in 777 homes. More than half (57%) of the homes experienced mold/water damage. For the homes that had visible mold on the building material, tape samples were taken from the contaminated surface. It was shown that 10% of homes with visible mold damage had *Alternaria* spores on the damaged surfaces. Cats and dogs were present in 19.7% and 31.1% of homes, respectively. More than 90% of homes had either carpet or area rug covering their floors. Antigen levels in homes between carpet and area rug showed non-significant (*Alternaria*, cat, dog, cockroach) or border line (house dust mite; $p = 0.05$) differences and therefore, results obtained form carpet and area rugs were grouped together in further analysis.

Table 2 shows the distribution of antigen levels measured from dust samples collected in the child's primary activity room. The percent of samples that were below the lower limit of detection were 10, 13, 8, 62, and 86% for *Alternaria*, cat, dog, house dust mite and cockroach, respectively. Antigen loading ($\mu g/m^2$) had higher variation than antigen concentration ($\mu g/g$), but was significantly correlated with antigen concentration level ($p<0.001$). The total amounts

of collected fine dust ranged from 0.005 to 7.985 g/m^2, and the median and mean were 0.33 and 0.62 g/m^2, respectively.

3.1. Reliability of home characteristics data and variability of allergen levels

Total of 42 factors were investigated to study the reliability of home characteristics data and classified into 26 home characteristics as listed in Table 1. Number of residents and floor material of the PAR were shown to be identical for both home visits. In contrast, data for the presence of stuffed toys in the PAR and opening windows for air circulation purpose was significantly different between the initial and repeat home visit ($p<0.05$). Thus, these home characteristics were omitted in the further statistical analyses in relationship with antigen level. The other home characteristic data were statistically the same.

Variability of dust allergen levels in different time points was estimated as an average of CVs obtained from 30 pairs of dust allergen levels. The average CVs (range) for *Alternaria*, cat, dog, house dust mite and cockroach were: 45 (0–121), 54 (0–124), 51 (0–126), 53 (0–140), and 40 (0–134)%. This variability includes the temporal variation in allergen levels as well as the variation caused by sampling and allergen assay.

3.2. Relationship between home characteristics and antigen level

Tables 3–7 show the association between different types of antigen levels and home characteristics obtained through the generalized multiple regression analysis. Among the investigated home characteristics, only those, which were significantly associated with either antigen concentration or loading, are included in the tables. Mean antigen levels in home with regard to various home characteristics and their sub categories were calculated, and relative risk (RR) was estimated for home characteristics that showed significant relationships with antigen levels. Among the investigated home characteristics the following had the highest number of associations with antigen levels: number of dogs, presence of indoor plants, and floor material.

The number of dogs was associated with the loading and concentration of *Alternaria*, cat, and dog allergen/antigen. The presence of indoor plants in the PAR showed association with the loading of *Alternaria*, cat, and cockroach and the concentration of *Alternaria* and cockroach. Floor material of PAR showed a relationship with the loading of *Alternaria*, cat, and dog allergen/antigen, and with the concentration of cockroach allergen. Thus, antigen loading was associated with home characteristics more often and more consistently compared to antigen concentration.

3.3. Alternaria antigen

Alternaria antigen levels in both concentration and loading were associated with several home characteristics: number of dogs, presence of indoor plants, season of home visit, and type of the PAR (bedroom or busy room with high level of physical activity such as living room, family room, kitchen, and dining room). *Alternaria* antigen level was significantly higher in homes that had two or more dogs whereas the presence of indoor plants was significantly associated with lower level of *Alternaria* antigen. *Alternaria* antigen level showed seasonal variation being highest in fall samples and lowest in spring samples. Bedrooms had lower *Alternaria* antigen levels than the other rooms.

The other home characteristics were significantly associated with *Alternaria* antigen in one measurement unit only. In antigen concentration, homes using a dehumidifier or clothes dryer venting into the home had significantly higher levels of *Alternaria* antigen than homes that did not have these features. The appearance of cockroaches was positively associated with concentration of *Alternaria* antigen. In antigen loading, homes that used any insect

extermination methods, or had a carpet or an area rug in the PAR had higher levels of *Alternaria* antigen than homes that did not use insect extermination or were non-carpeted, respectively. There was no significant association between the mold/water classes and the *Alternaria* antigen level in either concentration or loading unit.

3.4. Cat (Fel d 1)

Home characteristics associated with both cat allergen loading and concentration were the number of cats and dogs. Homes with more cats had significantly higher levels of cat allergen, but homes with dogs had lower levels of cat allergen. The number of dogs, however, showed positive, though not significant association with the number of cats (data not shown). Only allergen loading was associated with the following home characteristics: the presence of indoor plants, relative humidity, floor material, and vacuum frequency. Carpeted homes had higher cat allergen loading than non-carpeted homes. Vacuuming frequency was associated with allergen loading, but the relationship was nonlinear. Relative humidity had negative relationship with cat allergen loading. Small family size (four or less) was associated with higher allergen concentration.

3.5. Dog

The number of dogs was positively and the family size was negatively associated with both loading and concentration of dog antigen similarly as seen with the cat allergen. There was, however, no significant relationship between the number of residents and the ownership of cat or dog (data not shown). Additionally, samples collected in the fall exhibited significantly higher dog antigen loading compared to spring samples. Furthermore, carpeted homes had higher dog antigen loading than non-carpeted homes.

3.6. House dust mite

Only mold class was associated with both loading and concentration of house dust mite allergen. At the same time, however, the relationship was non-linear. The use of a dehumidifier was positively associated with allergen loading. Concentration of house dust mite allergen decreased with increased vacuuming frequency. Allergen concentrations in bedrooms were higher than those in other rooms. Use of a forced-air heating system was associated with lower concentration of house dust mite allergen.

3.7. Cockroach

Appearance of cockroaches was significantly associated with higher cockroach allergen loading and concentration. The presence of indoor plants was negatively associated with cockroach allergen in both loading and concentration. In addition, carpeted homes had significantly higher cockroach allergen concentrations than non-carpeted homes.

4. Discussion

This study showed that indoor antigens loadings as well as concentrations were associated with various home characteristics obtained from on-site home visits. In many epidemiological studies, antigen concentration has been investigated in the relationship with home characteristics, but loading was seldom used. In our study, larger numbers of associations were observed between home characteristics and antigen loading, and these associations were more consistent compared to antigen concentration. Moreover, antigen loading had larger variation between homes than antigen concentration indicating possibly larger differences in the children’s exposures.

In this study child's allergen exposure was assessed from floor dust samples. Despite the fact that infants spend most of their time in bed, a recent European study (Douwes et al., 2006) has shown association only between living area exposure and asthma in 4 years old children, but none with mattress exposure. This may be due to the fact that the main living room allergen levels for dust allergens were higher than that in the bedding in the infants' homes and because mattresses for infants were purchased new (use of less than 3 months) (Leaderer et al., 2002; Douwes et al., 2006).

The reliability in the home characteristics data was generally better for dichotomous data than continuous/semi-continuous data. The home characteristics showing poor data reliability (presence of stuffed toys and opening of windows) may not have meaningful relationships with antigen levels. It is not surprising to see variation in the presence of toys because people tend to move toys between different rooms. The difference in answer for open windows might be due to different weather at the time of home visits and different interviewees. Therefore, further statistical regression modeling was performed using only home characteristics with good reliability.

As expected, the level of cat, dog and cockroach allergen/antigen was significantly associated with the number of cat, dog, or appearance of cockroaches, respectively. This is in agreement with findings from other studies. Matheson et al. (2003) showed that acquiring a cat increased Fel d 1 in house dust about 900%. Furthermore, Arbes et al. (2004) and Gruchalla et al. (2005) demonstrated that cat and dog ownership has one of the largest influences on their allergen concentration (Fel d 1 and Can f 1) in house dust samples. Chew et al. (1998) showed that reported signs of cockroaches predicted high Bla g 1 concentration. In our study, the level of house dust mite allergen was significantly higher in bedrooms than in other investigated rooms. Bedding materials in bedrooms have been reported as a reservoir of house dust mite and its allergen (Dharmage et al., 1999; Arlian and Platts-Mills, 2001; Arlian et al., 2001; Matheson et al., 2003). As expected, increased vacuuming frequency decreased allergen levels but this was significant for only cat and house dust mite allergens.

The level of *Alternaria* antigen was associated with larger number of home characteristics than the other antigens. It was, however, not associated with visual mold/water damage, which could be one of the sources for the *Alternaria* antigen in homes. Many of the home characteristics associated with *Alternaria* affect the transport of particles from outdoors to indoors. *Alternaria* is one of the most common fungal genera outdoors (Shelton et al., 2002) and known to grow on rotting leaves and other biomaterial abundant in the fall (Platts-Mills and Solomon, 1993). Adhikari et al. (2005) reported that outdoor airborne *Alternaria* spore concentration in Cincinnati, OH peaks in fall. In this study, indoor *Alternaria* antigen level showed seasonal variation being highest in fall samples and lowest in spring samples. Therefore, elevated *Alternaria* antigen levels in fall dust samples may be explained by transport of outdoor *Alternaria* into the homes.

Having dogs, which showed significant association with the increase in *Alternaria* antigen level, might be another significant factor for the antigen transport. Most dogs in the CCAAPS homes were allowed to go out, and could carry outdoor allergens indoors on their feet and fur. Though we did not show that the number of family members was associated with higher *Alternaria* antigen level, others have shown the transport of cat and house dust mite allergens in human clothing and hair (Almqvist et al., 1999; Randall et al., 2005; Jackson et al., 2005; Karlsson and Renström, 2005). Salo et al. (2005) also found a positive association between *Alternaria* antigen concentration and the presence of dogs. Therefore, it is suspected that the outdoor activity of dogs increases the outdoor-to-indoor transport of *Alternaria* antigen. Higher *Alternaria* antigen levels in busy rooms with high activity during daytime compared to bedrooms may reflect higher activity as well as more traffic between outside and inside in

former type of rooms. Furthermore, *Alternaria* spores were found on mold-contaminated surface in only 10% of homes that had visible mold. Therefore, these associations suggest that the majority of *Alternaria* antigen in these homes may originate from the outdoor environment rather than from indoor mold/water damage.

Another group of home characteristics associated with *Alternaria* antigen was related with indoor microclimate. Homes, which had clothes dryers vented into living quarters or used dehumidifiers, showed higher *Alternaria* concentrations. Most homes using dehumidifiers had previously experienced excessive moisture inside the home and might have *Alternaria* growing in the carpet even though it was not visually observable at the surface. Similar observation was reported by Salo et al. (2005). Venting the cloth dryer into living quarters may be associated with increased moisture in the living quarters, which may also result in mold growth in the dust/carpet.

The level of house dust mite was also influenced by home characteristics related with indoor moisture: house dust mite allergen loadings were higher in homes using dehumidifier and in homes with no central forced air heating. Homes using a dehumidifier in this study already experienced excessive moisture, while homes using forced-air heating system are known to have warmer and drier environments, which are less favorable for house dust mite growth (Wickman et al., 1991). Other studies also reported positive relationship between relative humidity and house dust mite allergen (Cabrera et al., 1995; Arlian and Platts-Mills, 2001; Arlian et al., 2001), and negative association between usage of central forced air heating system and allergen level (Matheson et al., 2003; van Strien et al., 2004). House dust mite allergen level was highest in mold class 1 homes, which had minor mold/water damage. We did not, however, find direct association with the measured humidity and the mold class or the level of house dust mite allergen. The humidity record obtained during home visit was a snap shot of specific condition at that time, and did not represent long-term humidity condition, which is necessary information to examine indoor mold growth. Continuous monitoring of temperature and humidity would be needed to better characterize the indoor climate in future studies.

It is noted that the number of samples below LOD for house dust mite and cockroach in our study is somewhat larger than in a previous study (Mansour et al., 2001), who had 44% and 27% of samples below the limit of detection for house dust mite and cockroach allergen concentrations, respectively. In our study, 62% and 86% of the 777 homes had house dust mite and cockroach allergen levels lower than the detection limit, respectively.

Interestingly, the presence of indoor plants was negatively associated with the levels of *Alternaria* antigen as well as cockroach and cat allergen. It has been reported that plants can clean the air by collecting airborne particulates on their leaves, which offer large surface area for particulate deposition (Lohr and Pearson-Mims, 1996). Indoor plants, however, would be also reflective of lifestyle or other factors that in turn influence allergen level of *Alternaria*, cat and cockroach.

Carpeted floor was a significant risk factor for the increase in the loading of *Alternaria*, cat, and dog allergen/antigen and concentration of cockroach allergen. Similar findings have been reported by Foarde and Berry (2004) and Chew et al. (1998). Carpets or area rugs provide large surface areas and reservoirs for particle accumulation and microclimate for fungal growth. Thus, floor material is likely to affect amount of these antigens per unit floor area, which governs antigen exposure. For house dust mite, we did not find an association between house dust mite allergen and floor material, which differs from other studies that reported higher allergen level of house dust mite in carpeted than in non-carpeted homes [Chew et al., 1998 (Der f 1 or Der p 1); Mihrshahi et al., 2002 (Der p 1)].

Some of the associations found in this study are difficult to interpret and may be caused by analytical interferences in the antigen assays. For example, negative association was found between insect extermination and *Alternaria* antigen level. The chemicals used for insect extermination could interfere with the immunochemical assay as was also postulated by Chew et al. (1998). In conclusion, this study has investigated the relationships between home characteristics and antigen levels using both antigen loading and concentration. We revealed that antigen loading showed more consistent relationships with home characteristics than the traditionally used antigen concentration. Several home characteristics were newly investigated in association with antigen levels. Having indoor plants reduced antigen levels. Cat, dog and cockroach allergen/antigen levels were associated with the presence of cat, dog, and cockroaches in home. Therefore, the exposure to these antigens could be crudely estimated by accounting for the presence of the specific antigen source. *Alternaria* antigen was associated with larger number of home characteristics than the other antigens and may thus be more difficult to estimate based on solely home characteristic data. Factors affecting outdoor–indoor transport of fungal spores were more important determinants than visible mold for the level of *Alternaria* antigen. It supports that visual observation of mold damage alone is insufficient to assess fungal exposure.

Our findings provide information for future exposure assessment studies and can be used for home intervention with regard to allergen avoidance. Also we suggest the use of antigen loading in addition to antigen concentration as antigen level predictors for future exposure assessment.

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Table 1

Home characteristics and their prevalence (*n*=777 homes)

Home characteristics	Categories	*n*[a]	(%)[b]
Group 1(direct sources/factors or carriers of allergens)			
Mold classification	0	336	43.3
	1	403	51.9
	2	37	4.8
Appearance of cockroach	Yes	89	11.5
	No	688	88.5
Number of cats	0	607	80.3
	1	76	10.0
	≥2	73	9.7
Number of dogs	0	521	68.9
	1	179	23.7
	≥2	56	7.4
Number of residents	≤4	529	68.1
	>4	248	31.9
Number of stuffed toys in PAR[c]	0	325	42.3
	≥1	443	57.7
Number of indoor plants in PAR[c]	0	564	74.2
	≥1	196	25.8
Relative humidity in PAR (%)	<30	109	14.0
	30–50	587	75.6
	≥50	81	10.4
Temperature in PAR[c] (°F)	<75	433	55.7
	≥75	344	44.3
Season of dust sample collection	Spring	133	17.1
	Summer	240	30.9
	Fall	271	34.9
	Winter	133	17.1
Group 2(indirect sources or control factors of allergens)			
Insect extermination	Yes	263	33.9
	No	514	66.1
Distance of PAR from kitchen (ft)	≤14	308	47.1
	>14	346	52.9
Clothes dryer vented in living quarter	Yes	15	1.9
	No	762	98.1
Humidifier use	Yes	444	57.4
	No	329	42.6
Dehumidifier use	Yes	127	16.6
	No	639	83.4
Floor material in PAR[c]	Carpet or area rug	709	91.2
	No carpet/area rug	68	8.8
Vacuuming frequency	≤once/week	286	36.8
	2 times/week–once/day	371	47.8
	≥once/day	120	15.4
Vacuum type	Standard HEPA	474	69.1
		212	30.9
Group 3 (general housing characteristics)			
Area of homes (ft^2)	<1000	162	20.8
	1000–2000	270	34.8
	2000–3000	181	23.3
	3000–4000	106	13.6
	≥4000	58	7.5
Age of homes (year)	≤50	195	25.1
	>50	582	74.9
Building type	Apartment	164	21.7
	Attached house or row house	52	6.9
	Detached house	541	71.4
Location of PAR[c]	Busy room[d]	705	90.7
	Bedroom	72	9.3
Floor level of PAR[c]	0	21	2.7
	1	645	83.9
	≥2	103	13.4
Heating method	Forced air heating only	391	50.3
	Others	386	49.7
Air circulation method	Windows open	171	22.1
	A/C unit only	603	77.9
Dust amount per unit sampling area (g/m^2)[e]	≤0.3	388	49.9
	>0.3	389	50.1

[a] *n*: The number of homes with a particular characteristic. Numbers do not add up to 777 due to missing data.

[b] Percentages were calculated with available data and add up to 100.

[c] PAR: Primary activity room.

[d] Busy room: living room, family room, kitchen, and dining room.

[e] Not included in the regression analysis.

Table 2

Dustborne antigen levels in concentration (/g) and in loading (/m^2) for samples collected from the floor of child's primary activity room in 777 homes

	Number of analyzed samples (*n*)	Number of samples below lower limit of detection (*n*)	Unit	GM	Interquartile range	Correlation coefficient between concentration and loading ($p<0.001$)
Alternaria	774	77	µg/g	47.3	25.5–79.2	0.6
			µg/m^2	14.8	5.2–43.0	
Cat (Fel d 1)	775	98	µg/g	2.0	0.3–9.2	0.9
			µg/m^2	0.6	0.1–3.8	
Dog	775	62	µg/g	23.3	5.7–78.4	0.8
			µg/m^2	7.3	1.6–31.5	
House Dust Mite (Der f 1)	774	482	µg/g	0.5	0.1–0.9	0.8
			µg/m^2	0.2	0.04–0.5	
Cockroach (Bla g 1)	775	668	IU/g	0.8	0.4–0.9	0.6
			IU/m^2	0.2	0.1–0.7	

Table 3

Multiple regression model of the association between *Alternaria* antigen levels (concentration: μg/g and loading: μg/m^2) and home characteristics

Home characteristics	*Alternaria* (concentration)		*Alternaria* (loading)	
	Geometric mean (μg/g)	RR (95% CI)	Geometric mean (μg/m^2)	RR (95% CI)
Number of dogs				
No dog	45.6	1	13.8	1
One dog	47.1	0.97 (0.88, 1.07)	13.2	0.94 (0.84, 1.05)
Two or more dogs	65.7	1.25 (1.09, 1.44)	32.6	1.33 (1.14, 1.55)
Cockroach appearance				
No	47.5	1	14.8	
Yes	44.7	0.85 (0.75, 0.96)	13.0	
Plants in PAR				
No	48.3	1	16.4	1
Yes	44.1	0.90 (0.82, 0.98)	10.3	0.84 (0.76, 0.93)
Season				
Spring	28.6	1	6.7	1
Summer	36.7	1.21 (1.05, 1.40)	13.3	1.38 (1.20, 1.59)
Fall	76.1	2.01 (1.77, 2.28)	24.0	1.56 (1.36, 1.79)
Winter	46.5	1.42 (1.21, 1.66)	13.4	1.32 (1.13, 1.54)
Insect extermination				
No	44.7		12.4	1
Yes	52.2		19.5	1.14 (1.04, 1.24)
Use of a dryer				
No	46.9	1	14.4	
Yes	64.4	1.37 (1.06, 1.78)	22.8	
Use of a dehumidifier				
No	45.5	1	14.5	
Yes	56.6	1.14 (1.03, 1.26)	14.8	
Carpet				
No	49.2		5.0	1
Yes	47.0		16.1	1.35 (1.14, 1.46)
Type of room				
Busy room	48.0	1	14.9	1
Bedroom	39.6	0.78 (0.67, 0.91)	11.0	0.80 (0.69, 0.93)

RR: Relative risk. This measures the multiplicative change in mean allergen level compared to the baseline determined from the regression model. These are approximately the same as the empirical geometric means (same through Table 7).

CI: Confidence interval (same through Table 7).

Note: Home characteristics that show no value of RR were not significantly associated with allergen levels in a group-specific model and not included in the final model (same through Table 7).

Table 4

Multiple regression model of the association between cat (Fel d 1) allergen levels (concentration: μg/g and loading: μg/m^2) and home characteristics

Home characteristics	Cat (concentration)		Cat (loading)	
	Geometric mean (μg/g)	RR (95% CI)	Geometric mean (μg/m^2)	RR (95% CI)
Number of cats				
No cat	0.8	1	0.3	1
One cat	48.4	2.06 (1.90, 2.23)	14.9	2.12 (1.94, 2.31)
Two or more cats	108.1	2.18 (2.02, 2.37)	36.4	2.17 (2.00, 2.36)
Number of dogs				
No dog	2.2	1	0.7	1
One dog	1.6	0.85 (0.79, 0.92)	0.5	0.88 (0.82, 0.95)
Two or more dogs	1.7	0.81 (0.73, 0.91)	0.9	0.92 (0.81, 1.04)
Number of residents				
≤4	2.5	1	0.7	
>4	1.2	0.90 (0.85, 0.97)	0.4	
Plants in PAR				
No	1.8		0.6	1
Yes	2.3		0.5	0.88 (0.82, 0.95)
Relative humidity in PAR				
<30	2.3		0.7	1
30–50	2.1		0.7	0.94 (0.84, 1.04)
≥50	1.1		0.3	0.83 (0.72, 0.96)
Carpet				
No	0.9		0.1	1
Yes	2.1		0.7	1.28 (1.13, 1.45)
Vacuuming frequency				
≤once/week	2.9		0.9	1
2 times/week–once/day	1.6		0.5	0.91 (0.85, 0.97)
≥once/day	1.5		0.6	0.96 (0.87, 1.06)

Table 5

Multiple regression model of the association between dog antigen levels (concentration: μg/g and loading: μg/m^2) and home characteristics

Home characteristics	Dog (concentration)		Dog (loading)	
	Geometric mean (μg/g)	RR (95% CI)	Geometric mean (μg/m^2)	RR (95% CI)
Number of dogs				
No dog	11.0	1	3.4	1
One dog	122.1	1.82 (1.56, 2.13)	35.1	1.85 (1.72, 1.99)
Two or more dogs	157.4	1.74 (1.36, 2.23)	78.0	2.14 (1.94, 2.37)
Number of residents				
≤4	26.8	1	7.9	1
>4	17.0	0.86 (0.80, 0.93)	5.9	0.89 (0.83, 0.95)
Season				
Spring	17.4		4.1	1
Summer	22.9		8.4	1.15 (1.04, 1.27)
Fall	29.2		9.3	1.16 (1.05, 1.28)
Winter	19.9		5.8	1.03 (0.91, 1.15)
Carpet				
No	17.7		1.8	1
Yes	23.8		8.2	1.20 (1.05, 1.37)

Table 6

Multiple regression model of the association between house dust mite allergen (Der f 1) levels (concentration: μg/g and loading: μg/m^2) and home characteristics

Home characteristics	House dust mite (concentration)		House dust mite (loading)	
	Geometric mean (μg/g)	RR (95% CI)	Geometric mean (μg/m^2)	RR (95% CI)
Mold class				
0	0.4	1	0.1	1
1	0.6	1.22 (1.10, 1.36)	0.2	1.15 (1.04, 1.27)
2	0.5	1.0 (0.77, 1.29)	0.2	1.03 (0.81, 1.30)
Use of a dehumidifier				
No	0.5		0.2	1
Yes	0.6		0.2	1.14 (1.00, 1.29)
Vacuum frequency				
≤once/week	0.6	1	0.2	
2 times/week–once/day	0.4	0.90 (0.81, 1.01)	0.1	
≥once/day	0.4	0.83 (0.71, 0.96)	0.2	
Type of room				
Busy room	0.5	1	0.2	
Bedroom	0.6	1.24 (1.05, 1.46)	0.2	
Use of forced air heating				
No	0.5	1	0.2	
Yes	0.5	0.90 (0.81, 0.995)	0.2	

Table 7

Multiple regression model of the association between cockroach allergen (Bla g 1) levels (concentration: IU/g and loading: IU/m^2) and home characteristics

Home characteristics	Cockroach (concentration)		Cockroach (loading)	
	Geometric mean (IU/g)	RR (95% CI)	Geometric mean (IU/m^2)	RR (95% CI)
Cockroach appearance				
No	0.7	1	0.2	1
Yes	1.1	1.31 (1.16, 1.47)	0.3	1.27 (1.13, 1.43)
Plants in PAR				
No	0.8	1	0.3	1
Yes	0.7	0.89 (0.81, 0.98)	0.2	0.87 (0.79, 0.97)
Carpet				
No	0.7	1	0.1	
Yes	1.2	1.39 (1.20, 1.59)	0.3	