
Relationship of house-dust mite allergen exposure in children's bedrooms in infancy to bronchial hyperresponsiveness and asthma diagnosis by age 6 to 7

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Background: Several studies have suggested that exposure to house-dust mite (HDM) allergen in infancy increases the risk of developing asthma.

Objective: To determine whether exposure to higher levels of dust mite in infants increased the risk of developing bronchial hyperresponsiveness (BHR) or physician-diagnosed asthma by age 6 to 7 years.

Methods: A health maintenance organization-based cohort of 97 middle class suburban children born from 1987 to 1989 with a high cord blood immunoglobulin E, defined as ≥ 0.56 IU/mL, was followed as a part of the Childhood Allergy Study. During the first 2 years of life, monthly bedroom dust samples were collected and analyzed for Der f 1 and Der p 1. Between 6 and 7 years of age, 64 of the original cohort answered a questionnaire used to determine the presence of physician-diagnosed asthma, underwent clinical examination, skin prick testing, and methacholine inhalation challenge. Mann-Whitney tests were used to compare Der f 1 and Der p 1 levels in homes of children with and without BHR, and those with and without physician-diagnosed asthma.

Results: In all, 1,421 dust samples were collected and assayed. No significant differences were seen in either the mean, maximum, or minimum dust mite allergen levels in homes of children with versus without BHR, or children with versus without asthma. However, sensitization to HDM was associated with physician-diagnosed asthma ($P < 0.05$).

Conclusions: When compared with other studies, we were able to more accurately estimate the level of dust mite allergen exposure through repeated sampling over a relatively long period, incorporating seasonal variations. Although HDM sensitization and asthma were concurrently related, we were unable to find any relationship between level of HDM allergen exposure in children's bedrooms in early childhood and development of BHR or physician-diagnosed asthma by age 6 to 7 years.

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INTRODUCTION

The prevalence of asthma and allergic disease has increased in western society in recent decades.^{1,2} In view of this, environmental factors have been a primary focus of investigation, with indoor allergens, particularly house-dust mite (HDM), being proposed as a possible primary cause.^{3–6} Sporik et al³ found that exposure at age 1 to HDM allergen levels above 2 $\mu\text{g/g}$ was correlated with sensitization, whereas higher exposure levels of 10 $\mu\text{g/g}$ were associated with the development of asthma. Two other large prospective studies have been unable to support these findings.^{7,8}

Previous studies have based HDM allergen exposure on only one or two sample collections of household dust. The objective of our study was to determine the relationship of HDM allergen exposure in infancy measured serially at regular intervals to the later development of bronchial hyperresponsiveness (BHR) or physician-diagnosed asthma. A Detroit area population-based cohort of newborns was selected based on high cord blood immunoglobulin (Ig)E and followed intensely as a part of the Childhood Allergy Study until they were examined at age 6 to 7 years. Children who had high cord blood IgE trended toward being atopic by skin prick testing. This and other results have already been published.^{9–11} For the current analysis, we examined the association between HDM exposure in the first 2 years of life and the presence of methacholine hyperresponsiveness or physician-diagnosed asthma at age 6 to 7 years.

METHODS

Study Population

The Childhood Allergy Study is an ongoing study evaluating the environmental determinants of pediatric allergy and asthma. Selection of the study cohort has been previously

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described in detail.¹² In short, all women 18 years and older having prenatal visits in a geographically circumscribed suburban Detroit health maintenance organization (HMO) were invited to participate in the study. Eligibility requirements included being a member of the Health Alliance Plan HMO, having an estimated date of confinement between April 15, 1987 and August 31, 1989, delivery planned for 1 of the 3 hospitals serving the HMO patients, and residence within an area surrounding the clinics as defined by contiguous zip codes. Only one child per family was included in analyses. All aspects of the study were approved by the institutional Human Rights Committee and written informed consent was obtained at all appropriate times.

Cord blood was obtained at delivery and serum was separated and stored at -20°C until assay. Total IgE levels were quantitated as described previously by enzyme-linked immunoadsorbent assay.⁹ A cut point of ≥ 0.56 IU/mL was selected to define high versus low IgE categories based on a previous pilot study of 200 samples of cord blood that yielded a distribution of IgE in which the upper quartile was ≥ 0.56 IU/mL (unpublished data). Samples with initial results of >0.3 IU/mL were retested once or twice, and if at least 2 of the 3 results were ≥ 0.56 IU/mL, the level was classified as high.

The cohort of children with high cord blood IgE was followed intensively with monthly home visits during the first 2 years of life. Air and dust samples were obtained during each home visit. These children were invited for complete clinical evaluation at the age of 6 to 7 years.

Dust Samples

All dust samples were taken from the children's bedrooms. Der f 1 and Der p 1 were analyzed for purposes of this investigation. A detailed description of the collection and analysis of the dust samples has been previously described.¹³ Briefly, dust samples were obtained by vacuuming a 1-m^2 area of floor directly beside the child's bed for 2 minutes with a small vacuum fitted with a sampling attachment (Sample Vac, Quan-Tec-Air, Minneapolis, MN). To standardize the dust collection sampling, we selected one location for all the samples taken from all homes: the floor next to the infant's bed.

The dust samples were assayed for Der f 1 and Der p 1 using previously described monoclonal antibody-based assays (Indoor Biotechnologies, Chester, United Kingdom). Briefly, plastic microtiter plates were coated with appropriate monoclonal antibodies (5H8 for Der p 1 and 6A8 for Der f 1) in 50 mmol/L carbonate-bicarbonate buffer (pH 9.6) overnight. After coating, the plates were washed with phosphate-buffered saline containing 0.05% Tween 20 and then incubated for 1 hour with phosphate-buffered saline/Tween containing 0.05% bovine serum albumin. Dilutions of the standards or the unknowns were then added to duplicate wells in 50- μL aliquots and incubated overnight. After washing, the bound antigen was detected with biotin-labeled antibody, avidin-alkaline phosphatase, and phosphatase substrate (Sigma Chemical, St. Louis, MO). The ranges of the standard curves were from 1 to 125 ng/mL for Der p 1 and Der f 1. The

assay standards had been standardized at the University of Virginia by Dr. Martin Chapman. Interassay coefficients of variation were typically less than 12% for both assays. The units for HDM levels in dust were micrograms per gram of dust.

Clinical Evaluation

Between the sixth and seventh birthday, each child was invited to participate in a clinical evaluation by a single allergist. This evaluation included a standardized history and physical examination, pulmonary function tests, methacholine challenge, and skin prick tests for *Dermatophagoides farinae* and *Dermatophagoides pteronyssinus* (extracts from Bayer Biologics, Spokane, WA). Both positive (histamine 1 mg/mL) and negative (glycerosaline) controls were used. Tests were applied by the puncture method using a lancet (Bayer Biologics). A positive test was defined as a wheal of 2×2 mm or more with a flare larger than the wheal. If a subject had one or more positive skin tests, they were classified as atopic.

Asthma Diagnosis

A history of asthma was determined by asking the parent if the child had ever been evaluated by a physician as having asthma.

Methacholine Challenge

The methods used for spirometry and methacholine challenge have been described elsewhere.¹⁴ A positive methacholine challenge was defined as a decrease in forced expiratory volume in 1 second of 20% or more from the postsaline value after inhalation of methacholine up to 10 mg/mL concentration, and was used as the definition of BHR.

Statistical Methods

The two groups defined by BHR status (or asthma status) were compared for differences in various measures of allergen concentrations using a two-sample Wilcoxon test. The use of a nonparametric test was indicated by the highly skewed distributions of the allergen measures.

To be included in the analysis, an individual was required to have six or more observations. A mean, maximum, and minimum value was generated per individual over birth to 6 months and birth to 2 years. A P value <0.05 was considered as evidence of an effect.

The variable for skin test positivity (yes/no) was compared with BHR (and asthma) using a χ^2 test for differences in proportions.

RESULTS

In the Childhood Allergy Study, 953 of 1,194 women who were approached consented to the study. Cord bloods were not obtained in 106 deliveries for multiple reasons including miscarriage, stillbirth, delivery or medical complications, change of physician or hospital, or loss of a specimen. There were no differences between mothers refusing to participate or from whom cord blood samples were not obtained and the 847 women from whom cord blood was obtained at delivery with respect to age, race, and estimated date of confinement.

Table 1. Mean and Maximum Allergen Levels vs BHR

Age	Allergen	BHR positive (n = 19) Allergen level (s.d.) $\mu\text{g/g}$ dust	BHR negative (n = 45) Allergen level (s.d.) $\mu\text{g/g}$ dust	P value
6 months	Der f 1 mean	5.2 ($\pm$ 7.2)	7.3 ($\pm$ 9.9)	0.654
	Der f 1 max	12.5 ($\pm$ 16.1)	21.1 ($\pm$ 27.9)	0.551
	Der p 1 mean	1.7 ($\pm$ 3.9)	1.9 ($\pm$ 2.8)	0.055
	Der p 1 max	5.4 ($\pm$ 13.7)	5.8 ($\pm$ 7.3)	0.051
2 years	Der f 1 mean	3.77 ($\pm$ 4.76)	5.46 ($\pm$ 7.00)	0.552
	Der f 1 max	20.4 ($\pm$ 26.4)	30.7 ($\pm$ 33.0)	0.225
	Der p 1 mean	2.05 ($\pm$ 4.35)	1.72 ($\pm$ 2.83)	0.118
	Der p 1 max	15.5 ($\pm$ 17.3)	16.1 ($\pm$ 22.3)	0.589

Table 2. Mean and Maximum Allergen Levels vs Asthma

Age	Allergen	Asthma positive (n = 6) Allergen level (s.d.) $\mu\text{g/g}$ dust	Asthma negative (n = 59) Allergen level (s.d.) $\mu\text{g/g}$ dust	P value
6 months	Der f 1 mean	3.3 ($\pm$ 3.4)	7.0 ($\pm$ 9.5)	0.518
	Der f 1 max	7.2 ($\pm$ 6.7)	19.6 ($\pm$ 25.8)	0.347
	Der p 1 mean	0.4 ($\pm$ 0.2)	2.0 ($\pm$ 3.2)	0.063
	Der p 1 max	1.4 ($\pm$ 1.2)	6.1 ($\pm$ 9.9)	0.087
2 years	Der f 1 mean	4.45 ($\pm$ 4.92)	5.01 ($\pm$ 6.4)	0.856
	Der f 1 max	32.2 ($\pm$ 42.7)	27.7 ($\pm$ 30.5)	0.734
	Der p 1 mean	4.32 ($\pm$ 6.44)	1.68 ($\pm$ 2.99)	0.571
	Der p 1 max	64.8 ($\pm$ 100.7)	11.3 ($\pm$ 21.9)	0.910

The IgE level was ≥ 0.56 IU/mL in 147 of the 847 cord blood samples. Six of the samples with IgE ≥ 0.56 IU/mL also had an elevated IgA concentration (≥ 39 $\mu\text{g/mL}$) and were excluded because of the possibility of contamination with maternal blood, leaving a total of 141 samples in the high IgE group. A total of 107 children from the high cord blood IgE cohort enrolled in the intensive environmental followup, with 91 children having at least 6 and a maximum of 25 allergen measurements from the home during the sampling period (June 1987 to September 1991). Finally, 65 of the children underwent clinical examination at age 6 to 7 including history, physical examination, and skin prick testing, and constituted the cohort used for the current study. The average number of measurements per home for these children was 22.2 or 93% of the 24 planned measurements, for a total of 1,421 dust samples analyzed. Methacholine challenge was completed in 64 of the 65 children who returned for clinical evaluation.

BHR was present as defined by positive methacholine challenge in 19 of 64 children; however, only 6 of 65 children had been evaluated as having asthma by a physician. Level of HDM allergen exposure was not related to performance on baseline spirometry done before methacholine challenge (data not shown). There were no differences in mean or maximum levels of exposure to either HDM allergen in either the first 6 months or the first 2 years of life between the children with and without BHR (Table 1). Similarly, no significant differences were seen in mean or maximum HDM

allergen exposure between children with and without physician-diagnosed asthma (Table 2). Results of borderline statistical significance were in the direction of lower allergen exposure associated with BHR and asthma. Similar findings were seen for data on minimum exposure (data not shown).

The number of children with positive skin tests was 14 and 12 for Der f 1 and Der p 1, respectively. A positive skin test was associated with physician-diagnosed asthma ($P = 0.002$ for Der f 1 and 0.005 for Der p 1), but not BHR ($P = 0.622$ and $P = 0.390$).

DISCUSSION

In this group of patients, we were able to show that concurrent sensitivity to HDM, based on skin testing, is associated with physician-diagnosed asthma by age 6 to 7 years. Asthmatic patients exposed to high levels of allergen to which they have been sensitized have increased rates of current asthma⁴ and asthma exacerbation.¹⁵ Sporik et al¹⁵ found that the majority of children admitted to the hospital for asthma exacerbation were both sensitive to HDM and exposed to >10 μg Der p 1 per gram of dust. These facts are supportive of allergen exposure as a secondary cause of asthma.

However, primary causation is more difficult to prove, and our data did not confirm such a relationship in a random population. Our results differ from those previously published by Sporik et al.³ In that study, HDM exposure levels were based on a limited number of collections of dust from

the homes of children with a genetic risk for asthma or atopy. We were able to more accurately estimate the level of dust mite allergen exposure through repeated sampling over a relatively long period, incorporating seasonal variations. We used multiple measurements over the first 2 years of life to determine mean, minimum, and maximum levels of antigen exposure. Within the range of exposure studied, higher levels of exposure to HDM allergen during infancy was not related to the risk of BHR or physician-diagnosed asthma by age 6 to 7.

Our findings are consistent with at least two other large prospective studies.^{7,8} In the German Multicentre Allergy Study, early exposure to HDM, both at ages 6 months and 18 months, was not related to BHR at 7 years. In addition, there was no dose-response relationship of HDM exposure at 6 months to asthma at age 7 years, defined as "current wheeze," "wheeze ever," or "asthma diagnosed by a doctor."⁷ Burr et al⁷ found that HDM allergen levels in samples of dust obtained from infants' homes during the first year of life and at age 7 years were not significantly different between atopic wheezers and nonwheezers.⁸ Interestingly, we have found no association between the level of exposure to HDM in infancy and the risk for developing sensitivity to HDM or atopy in this birth cohort.⁹

A limitation of the study is that, because of cost considerations, only one location in a child's bedroom was sampled. In this case the carpet next to the child's bed. It may be said that the mattress would be a better source of dust sampling. However, Lau et al⁸ advocated the use of carpet rather than mattress dust sampling during the first years of life because it may be a better surrogate for overall exposure. More importantly, specific sensitization to dust mite allergen in their study was more strongly related to carpet than mattress allergen levels.⁸ No environmental controls or allergen avoidance were intended in the study and exposure to dust mite allergen in this study probably reflect real world level of exposure.

Finally, we found no association between positive skin tests to HDM and risk of BHR by age 6 to 7. On the surface, this would seem to be in opposition to our finding of HDM sensitization being associated with physician-diagnosed asthma. However, BHR determined by methacholine is not specific to asthma, and may include a small number of normal children.

CONCLUSION

Sensitivity to HDM appears to be associated with physician-diagnosed asthma. However, we were unable to show a correlation between level of HDM allergen exposure in children's bedrooms in early childhood and the subsequent development of asthma or BHR by age 6 to 7. This is significant considering we were able to very accurately determine level of exposure to HDM allergen through successful collection of

multiple samples at regular intervals over a 2-year period and incorporating seasonal variations.

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