Ara h2 levels in dust from homes of individuals with peanut allergy and individuals with peanut tolerance

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ABSTRACT

Background: Approximately 1% of the U.S. population has a peanut allergy. Previous studies that measured peanut protein in house dust support the hypothesis that household peanut consumption may lead to clinical sensitization through transdermal exposure.

Objective: The aim of this pilot study was to characterize Ara h2 levels in house dust from homes with and without individuals with peanut allergy.

Methods: Household dust was obtained from homes with an individual with peanut allergy and from homes with no individual with peanut allergy. Ara h2 levels were determined by using a monoclonal antibody–based immunoassay with a level of determination of 150 ng per gram of dust. Peanut consumption information was obtained by questionnaire.

Results: A total of 85 dust samples were collected: 38 from homes with a individual with peanut allergy and 47 from control homes. The median Ara h2 level in homes with an individual with peanut allergy was 1236 ng/g (interquartile range [IQR], 256–1342 ng/g), whereas the median Ara h2 level in homes without an individual with peanut allergy was 650 ng/g (IQR, 163–2201 ng/g). Ara h2 levels in dust from homes of individuals with peanut allergy were not significantly lower than in dust from control homes. Of the homes with an individual with peanut allergy, 15 reported complete avoidance of peanut in the home (39%). Ara h2 levels in homes that completely avoided peanuts were not significantly lower than Ara h2 levels in homes that did not restrict peanuts ($p = 0.531$).

Conclusion: Although families may restrict peanuts and peanut products in the home, there was still detectable Ara h2 levels found in homes. Each subject's definition of restriction may vary, there seemed to be peanut protein entering the home, although the protein origin is not known. Possibilities include cross-reactivity with another antigen or transport into the home on some vector. Further investigation of hypotheses regarding cross-reactivity and environmental exposure to Ara h2 is necessary.

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Peanut allergy is increasingly common in the U.S. childhood population. The estimated prevalence of school-age childhood peanut allergy based on either telephone surveys or specific immunoglobulin E (sIgE) levels and clinical information is between 2 and 4.6%.^{1,2} A randomized, cross-sectional survey of almost 40,000 households in the United States found that peanut allergy occurred in 2% of all children and 25% of children with food allergy.³ The

Centers for Disease Control and Prevention estimates that the prevalence of food allergy is growing ~1.8% per year among children ages <18 years.⁴ Currently, the recommended management of peanut allergy is strict avoidance of peanut.

The National Institute of Allergy and Infectious Disease recently released guidelines that recommend early introduction of peanuts. The expert panel recommends infants with severe eczema, egg allergy, or both have introduction of age-appropriate peanut containing food as early as 4–6 months of age to reduce the risk of peanut allergy. Those with mild-to-moderate or no eczema can begin introducing peanuts at approximately 6 months of age or when age appropriate.⁵ The dual allergen exposure hypothesis put forward by Du Toit *et al.*,⁶ is that early cutaneous exposure to food protein through a disrupted skin barrier leads to allergic sensitization, whereas early oral exposure to food allergen induces tolerance. This hypothesis relies heavily on the observation that peanut protein is present in house dust and on surfaces around the home.

Numerous studies indicate that peanut protein and specific peanut allergens can be measured in house

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dust from homes of infants and children,⁷⁻¹² and several of these studies indicate that peanut allergen development is enhanced in the presence of factors that would enhance skin exposure, such as atopic dermatitis and/or filaggrin mutation.^{7,9} The results of these studies indicate that complete peanut avoidance may be difficult if not impossible. Although, in any given home, the individual with peanut allergy avoids peanut ingestion, other individuals in the home may still consume peanuts. However, many households with a single individual with peanut allergy completely restrict all peanut products in the home. Whether or not restriction of peanuts and peanut products from the home will decrease Ara h2 levels remains unknown.

This study specifically looked at Ara h2 in the household dust of both individuals with peanut allergic and individuals with peanut tolerance. Although the impact of peanut consumption is known, it is not clear what impact exposure to Ara h2 in house dust will have on individuals with peanut allergy and if the variance in dust-borne Ara h2 affects an individual's peanut sensitization. In this study, we hypothesize that homes of children with peanut allergy will have lower levels of peanut allergen (Ara h2) in their house dust versus homes where there is no identified peanut allergy. We also hypothesize that households of individuals with peanut allergy that restrict peanuts will have even lower Ara h2 levels than homes without peanut restriction.

METHODS

Study Design

This was a prospective, cross-sectional study performed at a single center by using house dust collected from home vacuum cleaners. A previous study from this group showed comparable levels of environmental allergens in house dust collected from home vacuum cleaners versus other collection strategies.¹³ Participants with peanut allergy and participants who were nonallergic controls of all ages were recruited from the allergy clinic and the hospital intranet Web site. Peanut allergy was defined as a self-reported history of immediate onset of objective symptoms within 2 hours of eating peanut and/or as sIgE testing results to whole peanut of >0.35 kU/L. The control subjects were individuals from households without an individual with peanut allergy. The participants or household members who worked in a factory that processed peanuts were excluded from this study. Each participant or parent brought in one zip lock bag of house dust from the vacuum cleaner at home and also filled out a questionnaire that collected information regarding the subject's age, type of reaction, peanut consumption, type of home, and cleaning practices of the home. If sIgE to peanut and Ara h2 levels were

known, then this information was recorded. All the parents of the child or the participants if >18 years provided verbal informed consent, and our institutional review board approved this study with a waiver of written consent. This study was approved by the Children's Mercy Hospital Pediatric Institutional Review Board (approval 14110480).

Ara h 2 Assay

The house dust was sifted to remove gross debris and was extracted in a detergent medium. A monoclonal-based immunoassay specific for peanut allergen Ara h 2 (Indoor Biotechnologies, Inc. Charlottesville, VA) was used to determine the amount of Ara h2 protein in the dust sample. Peanut flour was used as a positive control. Extracts from many common aeroallergens found in house dust, including *Alternaria*, *Cladosporium*, dog, cat, and American roach were tested, and showed no cross-reaction (data not shown). The lower level of detection used was 150 ng Ara h2 per gram of house dust. All assays were performed as instructed by the manufacturer.

Statistical Analysis

Groups were compared by using the Mann-Whitney *U* test. All analyses were performed by using SPSS (IBM Corporation, Chicago, IL) or Excel (Microsoft Corporation, Redmond, WA). The study was designed with an adequate sample size to power an answer to the question of the difference in Ara h2 levels between groups with a confidence of 95%.

RESULTS

A total of 85 dust samples were collected: 38 from homes with an individual with peanut allergy and 47 from control homes. The median age of participants with peanut allergy was 8 years (interquartile range [IQR], 4–10 years). Further demographic aspects are presented in Table 1. The median Ara h2 level in homes with an individual with peanut allergy was 1236 ng/g (IQR, 256–1342 ng/g), whereas the median Ara h2 level in homes without an individual with peanut allergy was 650 ng/g (IQR, 163–2201 ng/g). The distribution of Ara h2 in control homes, homes with individuals with peanut allergy, and homes with individuals with peanut allergy where peanut consumption was strictly avoided is depicted in Fig. 1. Statistical analysis by the Mann-Whitney *U* test used to compare differences between two independent groups indicated no significant difference. Of the homes with an individual with peanut allergy, 15 reported complete avoidance of peanut in the home (39%). Ara h2 levels in homes that completely avoided peanuts were not significantly lower than Ara h2 levels in homes that did not restrict peanuts ($p = 0.531$).

Table 1 Characteristics of the study population

Demographic	Subjects with Peanut Allergy (<i>n</i> = 38)	Controls (<i>n</i> = 47)
Age, median (range), y	8 (2–55)	
Type of dwelling (<i>n</i> = 85), no.		
House	33	39
Apartment	2	3
Townhome	3	5
Vacuum frequency of the home (<i>n</i> = 85), no.		
Daily	6	6
Weekly	28	33
Biweekly	0	3
Monthly	4	5
Household peanut consumption in 1 wk, median (range), cups*	0.125 (0–3)	0.5 (0–4)
Households in which peanuts were restricted (<i>n</i> = 85), no.	15	1
Ara h2 levels in the house dust, median (IQR), ng/g	1236 (163–2201)	650 (256–1342)
Detectable IgE >–0.35 kU/L (<i>n</i> = 38), no.	30	
Detectable Ara h2 >0.35 kU/L (<i>n</i> = 38), no.	9	
IgE levels, median (IQR), kU/L	14.2 (3.53–62.65)	
Ara h2 levels, median (IQR), kU/L	7.46 (3.92–8.92)	
Reactions reported (<i>n</i> = 38), no.	32	
Anaphylaxis, no.	17	
Two systems involved	6	
GI only	1	
Skin only	8	
Unknown or never ingested, no.	6	

IQR = Interquartile range; IgE = immunoglobulin E; GI = gastrointestinal.

*In the peanut allergy group, 5 were unable to quantify the amount consumed, and, in the control group, 11 were unable to quantify the amount consumed.

The median peanut sIgE and the median sIgE directed toward Ara h2 in individuals who were peanut sensitized and participated in the study were 35.7 kU/L (range, <0.35 to >100 kU/L) (*n* = 31) and 7.5 kU/L (range, 1.6–28.9 kU/L) (*n* = 9), respectively. In the homes of individuals with peanut allergy, the median consumption of peanuts in 1 week was 0.125 cups (range, 0–3 cups); 39% of these homes restricted peanuts. In the control group, the median consumption was 0.5 cups (range, 0–4 cups), with one home reporting peanut restriction. Homes with an individual with peanut allergy reported significantly lower peanut consumption than control homes (*p* = 0.006).

DISCUSSION

Results of this study indicated that, not only is Ara h2 measurable in dust from homes that restrict peanut consumption, but, also, there is no significant difference in the amount of Ara h2 in vacuumed dust in

midwestern U.S. homes that strictly avoided peanut consumption compared with those that did not. Results of this study also indicated that reported avoidance of peanuts and peanut products in homes of individuals with peanut allergy did not significantly reduce the Ara h2 levels in house dust from midwestern U.S. homes. A previous U.S. study measured Ara h2 in inner city schools and bedrooms of patients with asthma.¹⁴ The researchers found a median level, of 0.76 µg/g, of detectable Ara h2 in school samples and a median level, of 1.13 µg/g, of Ara h2 in bedroom samples.¹⁴ These levels are comparable with what we found in the vacuumed house dust. The Learning Early About Peanut Allergy (LEAP) study also measured whole peanut protein on bed sheets in both peanut consumption and nonconsumption groups but not in relation to control subjects.¹⁵ The ranges reported in the LEAP study for whole peanut protein are consistent with the general ranges reported here for Ara h2 in house dust from Midwest U.S. homes, although

Figure 1. Levels of Ara h2 in the control homes versus peanut allergy homes (with and without restriction of peanut consumption). ○ and ★ represent outliers that were excluded from analysis.

we did not find the reported differences between consumption groups.

This study had a few weaknesses. It was performed in households from the Midwest United States. The consumption of peanuts may be different in both quantity and form of consumption from that in other parts of the world. Another weakness is the possibility of other proteins in house dust that might cross-react with the antibodies used in the assay and also act as cross-reactive sensitizers to Ara h2. We believed that this possibility was remote but could not be ruled out at this time. The assay was based on monoclonal antibody recognition of an epitope most likely unique to Ara h2. We tested a limited number of possible cross-reacting allergens and detected no interference with the assay. Modeling studies based on known protein allergens indicate different kinds of nut, *e.g.*, hazelnut, almond, walnut, cashew, pistachio, and pecan, will show no cross-reaction.¹⁶ Also, it has been reported that extracts from oat, corn, wheat, barley, sesame, or soy do not interfere with a polyclonal antibody-based assay for peanut protein.¹⁷

The interaction of peanut protein with the human immune system is clearly complex. Based on the transcutaneous sensitization hypothesis presented in studies by Brough *et al.*^{7-9,12} and the dual allergen exposure hypothesis presented by Lack,¹⁸ the content of peanut allergen in house dust could have great significance to the field of food allergy. Fox *et al.*,¹⁹ related weekly consumption of peanuts by all the family members during the first year of the life of a child to a higher risk

of that child having peanut allergy. Results of other studies showed a correlation between household consumption of peanut with total peanut protein in household dust⁸ and bed dust²⁰; however, a similar association between household peanut consumption with Ara h2 has not been fully demonstrated. Thus, our study raises several questions concerning previous findings in U.S. populations, including the question of whether Ara h2 correlates with peanut protein in house dust and what is the source for Ara h2 in house dust. Given that Ara h2 is the major allergen in peanut and that a previous study showed the importance of IgE to Ara h2 as a predictor of clinical peanut allergy,²¹ we suspect that Ara h2 in house dust may be important in sensitization, but all of these hypotheses require further study.

A published letter to the editor showed that a clear relationship was found between nut consumption by relatives and IgE-mediated sensitization in children <18 months old.²² In our study, we did not assess the age of diagnosis, but, in 40% of our restricted homes, the eating practices of family members changed after receiving a peanut allergy diagnosis. This could explain why individuals in our study had a peanut allergy despite no current consumption in the home. The strength of this study was the novel detection of Ara h2 in homes, although we did not test to identify if it was biologically active. Another limitation was that peanut allergy was defined by self-report and not by double-blind placebo controlled food challenge. It was thought that double-blind food challenge presented excessive risk in this

study. In addition, because the subjects brought dust from their home vacuum cleaners, variations may exist in the dust collected.

CONCLUSION

This study identified some critical questions that need further investigation, such as the origin of the Ara h2 if there is no peanut consumption in the home, the possibility that Ara h2 cross-reacts with a yet unidentified protein source and the biologic activity of Ara h2 in house dust. We could not support the hypothesis that homes of children with peanut allergy have lower levels of peanut allergen (Ara h2) in their house dust versus homes in which there is no identified peanut allergy. Nor could we support the hypothesis that households of individuals with peanut allergy that restrict peanuts have lower Ara h2 levels than homes without peanut restriction. Because this was a pilot study with its inherent limitations, further research is needed to confirm our findings and investigate the questions raised.

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