

ORIGINAL PAPER

Repeated measurements of mite and pet allergen levels in house dust over a time period of 8 years

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Clinical and Experimental Allergy

Summary

Background Studies of the association between indoor allergen exposure and the development of allergic diseases have often measured allergen exposure at one point in time.

Objective We investigated the variability of house dust mite (Der p 1, Der f 1) and cat (Fel d 1) allergen in Dutch homes over a period of 8 years.

Methods Data were obtained in the Dutch PIAMA birth cohort study. Dust from the child's mattress, the parents' mattress and the living room floor was collected at four points in time, when the child was 3 months, 4, 6 and 8 years old. Dust samples were analysed for Der p 1, Der f 1 and Fel d 1 by sandwich enzyme immuno assay.

Results Mite allergen concentrations for the child's mattress, the parents' mattress and the living room floor were moderately correlated between time-points. Agreement was better for cat allergen. For Der p 1 and Der f 1 on the child's mattress, the within-home variance was close to or smaller than the between-home variance in most cases. For Fel d 1, the within-home variance was almost always smaller than the between-home variance. Results were similar for allergen levels expressed per gram of dust and allergen levels expressed per square metre of the sampled surface. Variance ratios were smaller when samples were taken at shorter time intervals than at longer time intervals.

Conclusion Over a period of 4 years, mite and cat allergens measured in house dust are sufficiently stable to use single measurements with confidence in epidemiological studies. The within-home variance was larger when samples were taken 8 years apart so that over such long periods, repetition of sampling is recommended.

Keywords between-home variance, cat allergen, exposure, mite allergen, within-home variance

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Introduction

Several studies have investigated the association between indoor allergens and the development of allergic diseases [1–7]. Most of these studies used dust samples to estimate the allergen exposure taken at one point in time.

Few studies have examined the variability of indoor allergen levels over time. Most of these studies considered time periods of 1 or 2 years [8–12]. Two other studies investigated the variability of indoor levels over

time periods of 4 [13] and 6 [14] years. Peterson et al.[13] analysed 6–36 repeated measurements of air and dust samples obtained from the children's bedroom but did not report correlations between separate measurements. Topp et al.[14] analysed two repeated measurements of dust samples obtained from mattress and living room floor. Crude correlations ranged from 0.42 to 0.61 for mite allergens, for subjects still living in the same house after 6 years. Correlations for Fel d 1 were lower at 0.21–0.38.

The importance of establishing the ratio of within- to between-home variance of these types of measurements is that estimates of the associations between allergen exposure and (e.g.) asthma or atopy are drastically attenuated when the within-home variance is large compared with the between-home variance. This makes it more difficult to find associations when they exist, and it also leads to underestimation of exposure-response relationships [15, 16].

In this study, we used data from the PIAMA study to investigate the variability in dust concentrations of Der p 1, Der f 1 and Fel d 1 over a time period of 8 years. Two dust samples were taken from the parents' mattress, three from the living room floor and four from the child's mattress. The aim was to establish the variance within and between homes of these measurements over a period of 8 years.

Materials and methods

Study design and study population

The data used in this analysis were collected in the framework of the Dutch PIAMA birth cohort study. The PIAMA study started in 1996. It has a baseline population of 4146 children and consists of a natural history part and a double-blind placebo-controlled intervention part. Children were selected from the general population using a validated screening questionnaire [17]. In the intervention study, only children born to allergic mothers were included, and in the natural history part children born to allergic as well as non-allergic mothers were included. The aims of the PIAMA study are (a) to evaluate to what extent the use of mite-impermeable mattress covers can reduce the incidence of asthma and mite allergy and (b) to study the natural history of childhood allergic disease from birth in relation to several environmental and genetic risk factors. A detailed description of the study was published elsewhere [18].

One hundred children of the PIAMA population were randomly recruited to participate in this study according to the following three criteria: they did not move houses from birth until the age of 8 years; their homes were visited at the age of 3 months, 4 and 6 years to collect dust; and they were living in the middle or the eastern part of the Netherlands. These restrictions were applied because we wished to study the same homes as in previous years, because we wished to study children with complete data at ages 3 months, 4 and 6 years. For logistic reasons, the children living near the northern paediatric centre in Groningen were excluded from participation. The sampling scheme was governed by the requirements of the epidemiological study, emphasizing mattress samples early in life (as the baby bed and possibly the parental bed is the place where infants spend most of their time),

then adding living room floor samples at later stages. Sampling at ages 4 and 6 was determined by clinical investigations at age 4 and by participation in an international study that required sampling of about 350 homes of the study again at age 6 [1, 19]. The homes of the recruited children were visited for the fourth time at the age of 8 to collect dust from the child's mattress, the parents' mattress and the living room floor. Of those approached for participation, only one refused. The number of 100 children was chosen as this is sufficient to detect even relatively small correlations between repeated measurements with statistical significance – a correlation of about 0.28 is significant in a two-sided test at $P=0.05$ with a statistical power of 0.8 if variances of the repeated measurements are equal.

Dust samples

When the children were 3 months, 4 and 6 years old, dust samples were taken in their homes and analysed for Der p 1, Der f 1 and Fel d 1. The 100 selected children were visited again between November 2004 and February 2005. The child's mattress was sampled at all four time-points. The parents' mattress and the living room floor were sampled at two (3 months, 8 year) and three time-points (4, 6 and 8 years), respectively.

Dust sampling and extraction procedures have been described in detail in van Strien *et al.* [20]. Briefly, dust sampling of the child's mattress and the parents' mattress was performed after the blanket was removed. Dust was sampled for 2 min for the entire upper surface of the sheets, on which the subject actually slept. We did not remove the sheets as this would not represent actual sleeping conditions. With respect to the living room floor, an area of 1 m² (wall-to-wall carpet/rug) or 2 m² (smooth floor) was vacuumed for 2 min [20]. A 'smooth' floor was defined as a floor with no textile floor cover. As smooth floors usually have less dust per surface area, we chose to sample a larger surface. We did not attempt to sample the exact same surface of living rooms over the years as we would have had too many exceptions related to furniture rearrangements, and also because we argued it would be more appropriate to include the additional variation related to differences in sampling location [21] into our estimates of within-home variance. Similarly, we did not attempt to standardize for season, as this also would have produced artificially low estimates of within-home variance. The results of the PIAMA study up to age 1 have been published [20, 22].

Dust extracts were analysed for house dust mite allergens *Dermatophagoides pteronyssinus* (Der p 1) and *D. farinae* (Der f 1) and cat allergen (Fel d 1) using reagents for a sandwich enzyme immunoassay, purchased from Indoor Biotechnologies (Cardiff, UK). The lower limit of detection was 8 ng/mL for Der p 1, 6 ng/mL for Der f 1 and

0.4 mU/mL for Fel d 1. Samples with undetectable amounts of allergen were given a value of two-thirds of the detection limit because the distributions were log-normal. House dust mite allergen levels were expressed as nanogram per square metre of the sampled surface (ng/m²) and nanogram per gram of dust (ng/g). Cat allergen was expressed as milli-units per square metre of sampling surface (mU/m²) and milli-units per gram of dust (mU/g), with 1 U = 4 µg protein.

Statistical analysis

The normality of distribution for measured dust and log-transformed dust was evaluated by the Shapiro-Wilk's test for normality. Log-transformed values were used in the analysis as the non-transformed allergen concentrations showed a positive skewed distribution, whereas the log-transformed allergen concentrations had a distribution not significantly different from normal.

Mite allergen levels were adjusted for season using a correction factor described by van Strien et al. [20]. These correction factors were calculated using multiple linear regression analysis, with dummy variables for the different seasons (January–March, April–June, July–September and October–December). The months October, November and December (autumn) were used as the reference season. For Der p 1, the correction factor was 0.8 for winter, spring and summer as compared with autumn. For Der f 1, the correction factors were 0.8, 0.6 and 1.0 for winter, spring and summer as compared with autumn, respectively.

Analysis of variance (with a correction for period-effects) was used to calculate within- and between-home variance components and the ratio of within- to between-home variance. This ratio indicates whether the variation of the repeated measurements within a single home is greater (ratio > 1) or less than (ratio < 1) the variation of the measurements between different homes.

All analyses were performed for the child's mattress, the living room floor and the parents' mattress. Furthermore, as a sensitivity analysis, analyses of variance were performed for living rooms with a smooth floor covering and for living rooms with a wall-to-wall carpet separately, for children with the same mattress since the age of 4 and for households without a cat.

Statistical analyses were performed using SAS statistical software (SAS, version 8.2, SAS Institute, Cary, NC, USA). *P*-values below 0.05 were considered to be statistically significant.

Results

The distributions of the allergen levels for the child's mattress, the parents' mattress and the living room floor are shown in Fig. 1 as box plots. For Der p 1 and Der f 1, the medians of the distributions of allergens found on the

child's mattress and the parents' mattress were the lowest for the visit at age 3 months. For the living room floor, the medians of the distributions were the lowest for the visit at age 8 years. With respect to Fel d 1, there was less variation in the medians for the different visits. Furthermore, there was a wide range in minimum and maximum value for all allergens.

Child's mattress

Table 1 shows the variance ratios calculated for the child's mattress. It is clear that for the mite allergens, the within-home variance was considerably larger than the between-home variance in each analysis that compared samples taken from the child's mattress at 3 months of age with samples taken at later ages. However, within-home variance was similar to between-home variance when samples were analysed taken at ages 4 and 6 and 4 and 8, respectively, and the ratio of within- to between-home variance was much smaller than unity when samples taken at ages 6 and 8 were considered. For cat allergen, variance ratios were close to unity in all analyses comparing samples taken at 3 months of age with samples taken at later ages, and clearly below unity when samples taken at ages 4, 6 and 8 years were considered.

Parents' mattress

Table 2 shows the variance ratios for the parental mattresses that were sampled at ages 3 months and 8 years only. Variance ratios were close to unity for Der p 1 and Fel d 1, but larger for Der f 1.

Living room floor

Table 3 shows the variance ratios for the living room floors. The results for the mite allergens showed that within-home variance was generally larger than between-home variance, whereas for cat allergen, the ratios were clearly below unity as for the child mattresses sampled at the same time-points.

Sensitivity analyses

The results for children who had the same mattress at ages 4, 6 and 8 were similar to those of the group as a whole (results not shown). Variance ratios were generally well below unity for Der p 1 and Fel d 1 on carpeted floors but not for smooth floors (Table 4).

Discussion

This study shows that for mite and cat allergen, the variance within homes is generally small compared with the variance between homes between ages 4 and years.

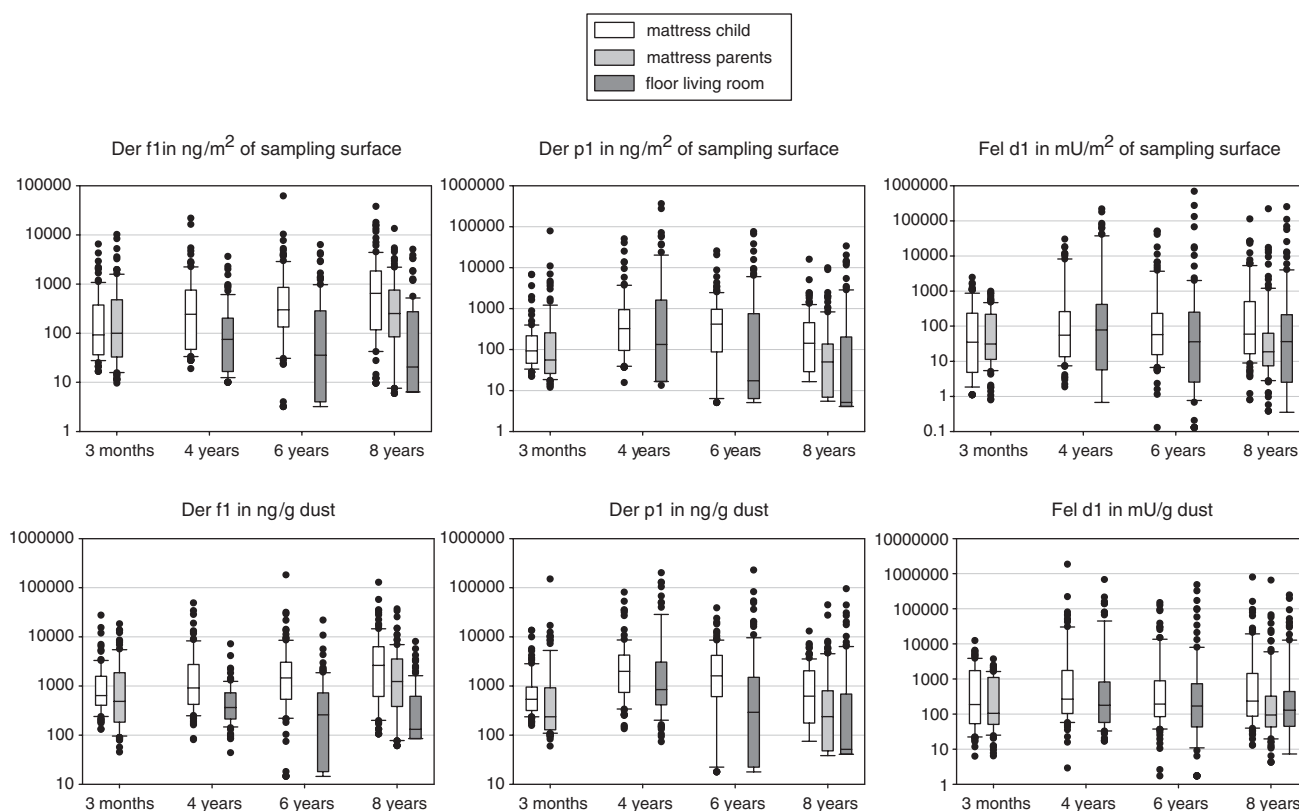


Fig. 1. Distribution of Der p 1, Der f 1 and Fel d 1 levels in dust samples from the child's mattress, the parents' mattress and the living room floor taken at ages 3 months and 4, 6 and 8 years. The box plots show the medians; the range of values between the 25th and 75th percentiles of the distributions; the whiskers show the range of values between the 10th and the 90th percentiles of the distributions; and the dots show the values outside the 10th and 90th percentiles of the distributions.

Table 1. Ratio of within- to between-home variances for allergens measured on the child's mattress; bold-type face dust per gram of dust, italic-type face dust per m² of sampling surface

Samples taken at		Ratio of within- to between-home variance			
		3 months	4 years	6 years	8 years
Der p 1	3 months		2.29	3.50	4.60
	4 years	2.45		1.00	0.64
	6 years	2.92	1.09		0.70
	8 years	2.80	0.96	0.73	
Der f 1	3 months		2.50	2.18	2.55
	4 years	1.25		1.10	0.90
	6 years	1.55	1.00		0.48
	8 years	2.11	1.23	0.57	
Fel d 1	3 months		0.91	1.02	0.84
	4 years	0.98		0.36	0.40
	6 years	1.27	0.50		0.40
	8 years	1.17	0.85	0.46	

Over a period of 8 years, the ratio of within- to between-home variance on child and parental mattresses is higher. The ratio of within- to between-home variance was generally less for cat allergen than for Der p 1 and the ratios for Der p 1 were less than for Der f 1.

Table 2. Ratio of within- to between-home variance for allergens measured on the parents' mattress when the child was 3 months, and 8 years old, respectively

	Ratio of within- to between-home variance
Der p 1 (g)	0.93
Der p 1 (m ²)	0.91
Der f 1 (g)	1.56
Der f 1 (m ²)	1.86
Fel d 1 (g)	0.93
Fel d 1 (m ²)	1.02

With respect to the child's mattress, the results for the sample taken at 3 months and the samples taken at ages 4, 6 and 8 years were different for all allergens. It is difficult to make a good comparison between the allergen concentrations for the child's mattress at the visits at 3 months of age and the allergen concentrations at the other visits, because of the large percentage of non-detectable allergen levels in the child's mattress dust at age 3 months. There are two explanations for the low allergen levels. First, one-third of the children had a new mattress at the time of birth [20]. Second, the dust samples were mostly taken in

Table 3. Within-home correlations and variances for living room floor; bold-type face dust per gram of dust, italic-type face dust per square metre of sampling surface

	Samples taken at (years)	Ratio of within- to between-home variance(years)		
		4	6	8
Der p 1	4		1.23	0.81
	6	<i>0.81</i>		2.22
	8	<i>0.84</i>	<i>1.53</i>	
Der f 1	4		4.71	1.75
	6	<i>1.39</i>		2.27
	8	<i>1.63</i>	<i>1.15</i>	
Fel d 1	4		0.68	0.70
	6	<i>0.82</i>		0.55
	8	<i>0.78</i>	<i>0.65</i>	

Table 4. Sensitivity analysis by living room floor type; bold-type face dust per gram of dust, italic-type face dust per square metre of sampling surface

	Floor type	Samples taken at (years)	Ratio of within- to between-home variance (years)		
			4	6	8
Der p 1	Carpeted	4		0.55	0.84
		6	<i>0.63</i>		0.85
		8	<i>1.03</i>	<i>0.91</i>	
Der f 1		4		1.50	1.08
		6	<i>1.40</i>		1.12
		8	<i>1.75</i>	<i>0.97</i>	
Fel d 1		4		0.58	0.72
		6	<i>0.60</i>		0.49
		8	<i>0.84</i>	<i>0.62</i>	
Der p 1	Smooth	4		17.33	1.67
		6	<i>10.50</i>		20.33
		8	<i>5.00</i>	<i>-200</i>	
Der f 1		4		8.20	11.00
		6	<i>6.00</i>		4.50
		8	<i>4.00</i>	<i>9.50</i>	
Fel d 1		4		0.61	0.39
		6	<i>0.88</i>		0.66
		8	<i>0.49</i>	<i>0.77</i>	

the winters of 1995/1996 and 1996/1997. During these winters, the temperatures were unseasonably low in the Netherlands, which likely decreased the amount of dust mites in homes [23]. All children moved to another, larger mattress usually before age 4, which adds to the explanation of the higher variance ratios using samples taken at 3 months and 4 years, than using samples taken at 4 and 8 years.

The results for cat allergen were generally more favourable than those for house dust mite allergen. The best predictors for Fel d 1 levels in a home are having a cat or

second-hand cat exposure [24, 25]. Homes without a cat often have detectable cat allergen levels due to residual allergen from previous cats in the home and/or allergen shed from clothing of visitors or from occupants who have visited homes with cats [26, 27]. However, cat allergen levels in homes with cats are much higher than levels in homes without a cat [28]. Fel d 1 is a relatively stable molecule and is generally bound to particles $< 2.5 \mu\text{m}$, which become airborne with minimal disturbance in the room and remain airborne for long periods. It sticks to walls, clothing, shoes, carpets, bedding and furniture, and it is difficult to get rid of completely, even after removing the cat from the home. Thus, a high correlation between repeated measurements of cat allergens is plausible [26, 27].

The sensitivity analysis for children with the same mattress showed the same trend compared with the analysis for all children. This was expected because for mattress dust, it has been shown that the age of the mattress contributes to higher allergen concentrations [29–31]. House dust mite allergen concentrations were related to indoor humidity, type of bedroom floor, frequency of vacuuming and age of the house. In the Netherlands, floor cover has been shown to be the dominant determinant of mite allergen levels [31, 32] with 6–12-fold differences between carpeted and smooth floors, compared with the effects of other factors such as vacuuming frequency being in the order of 2–3-fold. One study found a relation between the presence of a dog and higher levels of Der p 1 and Der f 1 in mattress dust [30].

The variance ratios for floors with a smooth cover were much higher compared with the variance ratios for carpeted floors. This can be explained by the observation that the major predictive variable for house dust mite allergen on floors is the type of flooring [31]. Dust from floors with a smooth cover has a concentration of house dust mite allergen six to 14 times lower than dust from carpeted floors [31].

Few studies have analysed repeated measurements of indoor allergens. Kuehr et al. [12] analysed repeated measurements of house dust mite allergen to investigate the year-to-year variation in dust from mattresses. Heinrich et al. [8] analysed repeated measurements of house dust mite allergens from living room floor over a time period of approximately 7 months. Both concluded that mite antigen allergen levels in house dust were a stable proxy for exposure over the relatively short periods of time in their investigations. Hirsch et al. [9] investigated the variability of house dust mite allergens within 3–24 months and found, as we did, that the within-home variance for Der p 1 and Der f 1 on carpets and mattresses was less than the between-home variance. Peterson et al. [13] analysed 6–36 repeated measurements of air and dust samples obtained from the children's bedroom but did not report correlations between separate

measurements, except for maximum correlations between pairs of measurements from the same homes, which ranged from 0.67 to 0.87 for cat and mite allergen. However, these numbers are not directly comparable to those reported in the other papers. Topp *et al.* [14] analysed repeated measurements of house dust mite allergens and pet allergens over a period of 6 years. Consistent with this study, they found variance ratios at or below unity for cat allergen. In contrast to our findings, the ratios were below unity for Der f 1 but not Der p 1, which may be explained by the fact that Der f 1 is a more common allergen in Germany than Der p 1.

As a word of caution, we need to stress that the results as presented in this paper apply to the population of homes that was studied. The sensitivity analysis by floor type shows, for instance, that a different result would have been obtained in a group of homes having smooth floors only. As a rule, decisions on optimal sampling frequency therefore need to be based on study-specific estimates of the within- and between-home components of variance.

Conclusion

We conclude that over a period of 4 years, repeated measurements of mite and cat allergens in house dust show adequate stability, as is evident from within-home variances, which were mostly smaller than between-home variances. Samples taken 8 years apart showed higher within-home variability relative to the between-home variability. As a single sample represents exposure over at least the 4–8-year age period well, there is no need to collect repeated samples over extended periods of time for use in epidemiological studies of the relationship between mite and cat allergen and allergic disease in children of this age.

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