ERJ Express. Published on March 1, 2007 as doi: 10.1183/09031936.00118806

Title: Bacteria and mould components in house dust and children's allergic sensitization

Short title: Bacteria, moulds and allergic sensitization

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Word count abstract: 197 words

Word count introduction through discussion: 4,020words

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Abstract

It has been suggested that early childhood exposure to microbial agents decreases the risk of allergies in children. We studied the association between microbial agents in house dust and allergic sensitization at age 2-4 years.

We performed nested case-control studies within ongoing birth cohort studies in Germany, The Netherlands, and Sweden and selected approximately 180 sensitized and 180 non-sensitized children per country. We measured levels of bacterial endotoxin, ß(1→3)glucans, and fungal extracellular polysaccharides (EPS) in dust samples from the children's mattresses and the living room floors.

Combined across countries, higher amounts of mattress dust and higher mattress dust loads of endotoxin, ß(1→3)-glucans, and EPS were associated with a significantly decreased risk of sensitization to inhalant allergens. After mutual adjustment, only the protective effect of the amount of mattress dust remained significant [odds ratio(95% confidence interval) 0.57(0.39-0.84)].

Higher amounts of mattress dust may decrease the risk of allergic sensitization to inhalant allergens. The effect might be partly attributable to endotoxin, ß(1→3)-glucans, and EPS, but could also reflect (additional) protective effects of (microbial) agents other than the ones measured. It is not possible to distinguish with certainty, which component relates to the effect, since their levels are highly correlated.

Key words: allergy, endotoxin, house dust, moulds, sensitization

Introduction

In the context of the hygiene hypothesis, early life exposure to microbial products is considered to play a major role in the development of asthma and allergies. However, it is not clear yet, which agents account for the proposed mechanism. Of all microbial products, endotoxin has been studied most extensively. Several authors have consistently found that exposure to elevated levels of house dust endotoxin decreases the risk of allergic sensitization in pre-school and school children. [1-3]

However, endotoxin is only one of the components of house dust with immune-stimulatory properties. Its advantage over other components is that there are well-established analytical methods available that can be applied in large epidemiological studies. Besides endotoxin, there is a variety of other microbial agents which are known to have immune stimulatory properties, such as ß(1→3)-glucans [4;5] bacterial DNA [6] and other bacterial components. [7;8] ß(1→3)-glucans are glucose polymers present in the cell wall of most fungi and yeasts, some bacteria, and vegetable materials; they have been measured as a marker of mould exposure in field studies. Moulds often grow together with different bacteria, and hence levels of ß(1→3)-glucan in house dust have been found to be highly correlated with endotoxin levels. [9] Thus endotoxin could be a marker for a broader range of microbial exposures.

We performed a study on the effects of outdoor and indoor air pollution on the development of allergic disease in children (AIRALLERG) and measured levels of endotoxin, ß(1→3)-glucans and extracellular polysaccharides (EPS) from *Aspergillus* and *Penicillium spp*. as another marker for mould exposure [10] in dust samples from approximately 1,000 German, Dutch, and Swedish pre-school and school children and

looked at its association with the allergic sensitization of the children at age 2-4 years. Furthermore, we followed Douwes et al. [11] and looked at the effects of the amount of sampled dust as a proxy of microbial exposure in general.

Materials and methods

Study design and study population

The study was designed as a nested case-control study within four birth cohort studies conducted in Munich and surrounding communities, Germany (GINI and LISA [12;13]), in the North, center, and Southwest of The Netherlands (PIAMA [14]) and the central and Northern part of Stockholm, Sweden (BAMSE [15]). The GINI study and part of the PIAMA study were designed as an intervention study studying the effect of different hydrolyzed formulas [12] respectively the use of mite-impermeable mattress and pillow covers [14] on the development of allergies and asthma in children with parental allergy and children with and without allergic mothers, respectively.

From each cohort (in Germany from the two cohorts combined) approximately 180 children with sensitization to common food and inhalant allergens at age 2-4 years were selected plus a random sample of approximately 180 non-sensitized children. Since the focus of the study was on inhalant allergies and inhaled exposure, the design was to select all children sensitized to inhalant allergens first. If there were not enough children (which was the case in all three countries), then children sensitized to food allergens were added. The children were visited for house dust collection at ages 5 (Germany and The Netherlands) and 7 years (Sweden), which was 16-55, 6-30, and 14-55 months after assessment of sensitization. In Germany and The Netherlands, the

sensitized children represent all eligible sensitized children whose parents were willing to participate; in Sweden, where the number of children with sensitization data available was larger than in the two other countries, sensitized children represent all eligible children with sensitization to inhalant allergens plus a random sample of children sensitized to food allergens whose parents were willing to participate. This results in a lower proportion of children with sensitization to food allergens only in Sweden compared to the other two countries. Families should not have moved between 6 months prior to blood collection for the IgE measurements and the AIRALLERG home visits. However, in Germany it was not possible to strictly follow this criterion; only 76% of the German participants fulfilled the not-moving criterion.

Definition of allergic sensitization

Within the original cohort studies, blood samples were taken when the children were 2 years (LISA), 3 years (GINI) and 4 years old (PIAMA and BAMSE). Immunoglobuline (Ig) E antibodies to common food and inhalant allergens were determined by a Pharmacia CAP system (Pharmacia, Uppsala, Sweden) in Germany and Sweden and by a Radio Allergo Sorbent test according the SOP used at Sanquin Amsterdam (Amsterdam, The Netherlands) in the Netherlands. Allergen panels differed between the cohorts, but specific IgE to egg white, milk, house dust mites, cat, tree and grass pollens were measured in all cohorts (Table 1). Allergic sensitization to any allergen was defined as specific IgE antibodies of at least 0.35 kU/L for one of the allergens tested. Allergic sensitization to inhalant and food allergens was defined as specific IgE antibodies of at least 0.35 kU/L for one of the inhalant respectively food allergens tested.

Dust collection

Between January 2002 and May 2003, in the months October to May, we visited a total of 358 (Germany), 347 (The Netherlands), and 364 (Sweden) children. During the home visit, we collected two house dust samples on the child's mattress and the living room floor by vacuuming the entire mattress surface area and either 1 m^2 of rugs > $4m^2$ and wall-to-wall carpets or 2 m^2 of smooth floors (if no carpets > 4 m^2 were present) for 2 minutes according to a standardized protocol using vacuum cleaners equipped with special nozzles (ALK allergen mouthpiece, Hørsholm, Denmark) to collect the dust on glass fiber filters (Schleicher & Schuell; ref. No. 370104). Dust samples were stored at –20^{o}C until extraction, and sent on dry ice to Utrecht (The Netherlands) for extraction and analysis.

Dust extraction and analysis

Dust samples were not sieved. Dust including filters was extracted sequentially as described previously. [16] The first supernatant was used to measure endotoxin by a chromogenic kinetic Limulus Amoebocyte Lysate test. [17] The second supernatant was used to measure EPS of *Aspergillus* and *Penicillium spp* by a sandwich Enzyme Immuno Assay. [10] ß(1→3)-glucan was measured in the third supernatant with a (ß(1→3)-glucan-specific inhibition enzyme immunoassay. [18] Furthermore, dust extracts were analyzed 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 sandwich enzyme immuno assays purchased from Indoor Biotechnologies (Cardiff, UK) as described

previously. [19] Associations between sensitization and exposure to allergens are beyond the scope of this article and will not be discussed. However, we will explore whether associations between allergic sensitization and exposure to bacteria and mould components were confounded by allergen exposure. Exposures were expressed both per gram of sampled dust (as a measure of concentration) and per square meter of sampling surface area (as a measure of the total burden or statistically speaking the interaction between the amount of dust and concentration of the respective biocontaminant). Samples with non-detectable amounts of endotoxin , ß(1→3)glucan, and EPS were assigned a value of two-thirds of the lowest detectable value.

Questionnaires

Questionnaire data collected within the original birth cohort studies was used to define potential confounding variables such as sex, parental allergy, and parental education. Confounding variables were defined as similar as possible given the information that was available.

Statistical analysis

Biocontaminant concentrations were log-normally distributed. Mean values were expressed as geometric means (GM) with a geometric standard deviation (GSD). Correlations were expressed as Pearson correlation coefficients based on natural-log (ln) transformed data. Nonparametric loess smoothers [20] (S-Plus 6.0, Insightful Corporation, Seattle, WA, USA) were used to investigate the functional relationship between allergic sensitization and microbial exposure. Since relationships were

generally log-linear, a parametric approach with ln-transformed exposure levels was applied in subsequent analyses. We first used standard logistic regression to analyze the association between microbial exposure and the binary outcome of sensitization to any allergen. We then analyzed the associations between microbial exposure and sensitization to food and inhalant allergens using polytomous logistic regression models [21] with four nominal response categories (sensitized to inhalant allergens only/sensitized to inhalant and food allergens/sensitized to food allergens only/non-sensitized). Since there was no difference in exposure-response-relationship between children sensitized to inhalant allergens only and children sensitized to inhalant and food allergens, these two response categories were combined in the final analysis. Results are presented as adjusted country-specific and combined odds ratios (from meta-analyses). Odds ratios were calculated using the same change in exposure for all countries rather than country-specific changes in exposure to ensure comparability of odds ratios between countries. The change in exposure was defined as the average country-specific interquartile range increase in exposure. In case of heterogeneity of effects between countries ($p<0.10$), the random effects approach described in Der Simonian and Laird [22] was used to calculate combined odds ratios. Statistical significance was defined by a two-sided α-level of 5%. Calculations were performed using the statistical Statistical Analysis System (SAS 9.1, Cary, NC, USA).

Results

Study population

The study population for the present analysis consists of 356 German, 338 Dutch, and 358 Swedish children with sensitization status and complete information on microbial exposure. Table 2 gives characteristics of the study population along with frequency distributions for allergic sensitization. Differences between countries are largely due to differences in the design of the original cohorts and in selection of the AIRALLERG participants. Boys (Germany and Netherlands only), children from allergic parents and children from parents with high education were over-represented compared to the original cohorts due to higher sensitization and/or participation rates among these groups (data not shown). Time lag between assessment of allergic sensitization and dust and season of dust collection did not differ significantly between cases and controls.

Amount of dust sampled, endotoxin, ß(1→3)-glucan, and EPS levels

Geometric means and geometric standard deviations of amount of dust sampled and microbial agents levels in the child's mattress dust and living room floor dust are presented in Table 3. Patterns between mattress and floor differ between countries. There were weak or no correlations between amounts of mattress dust and amounts of living room floor dust and mattress dust and living room floor dust biocontaminant levels, respectively ($r \leq 0.22$, data not shown). Endotoxin, ß(1→3)-glucan, and EPS loads of mattress dust were moderately to highly correlated with the amount of dust sampled (Table 4). Weak or no correlations were found between mattress dust

concentrations of endotoxin, ß(1→3)-glucan, and EPS and amount of dust sampled. Mattress dust endotoxin, ß(1→3)-glucan, and EPS loads were moderately correlated with each other, while there were weak or no correlations between concentrations. Similar but generally stronger correlations were found for living room floor dust samples (Table 4).

Associations between exposure to microbial agents and allergic sensitization

We found negative associations between amounts of dust sampled from the children's mattresses, endotoxin and ß(1→3)-glucan loads and sensitization to any allergen (Figure 1). Effects were homogeneous ($p>0.10$) between countries and statistically significant ($p<0.05$) when combined across countries. Effects became stronger when the cut-off for sensitization was increased [combined adjusted OR(95% CI) using 0.35 kU/L, 0.7 kU/L and 3.5 kU/L as cut-off for sensitization were 0.81(0.68-0.95), 0.75(0.63-0.90), and 0.64(0.51-0.81) for amount of dust; 0.82(0.73-0.93), 0.76(0.67-0.87), and 0.69(0.58-0.81) for endotoxin loads and 0.81(0.71-0.93), 0.75(0.65-0.87), and 0.67(0.56-0.81) for ß(1→3)-glucan loads]. No association was seen with EPS loads and for microbial agents expressed per gram of dust. When we looked into the effects of microbial agents on sensitization to food and inhalant allergens separately, we found effects limited to sensitization to inhalant allergens (Figure 2). In addition, there was a negative association between EPS loads and sensitization to inhalant allergens. No association was found for sensitization to food allergens. Additional adjustment for use of mite-impermeable mattress covers, pet-ownership, breastfeeding, and mite and cat allergen levels in mattress dust did not change the association considerably (maximum change in

odds ratio was 6%, data not shown). We did not adjust for age since age at blood collection is very homogeneous within cohorts except for Germany due to combination of two cohorts were blood was collected at age 2 an 3 years, respectively. To account for age and other differences between the two cohorts, models for Germany include a variable 'study design'.

No association was found between sensitization to inhalant allergens and amounts of dust sampled from living room floors [combined adjusted OR(95% CI) 0.93(0.58-1.48) per factor 10.6 increase in amount of dust per m^2], as well as living room floor dust endotoxin, ß(1→3)-glucan and EPS loads [combined adjusted OR (95% CI) 0.92(0.64-1.30), 0.91(0.56-1.47), and 0.93(0.64-1.37) per factor 15.4, 13.0 and 79.7 increase in exposure, respectively] and concentrations [combined adjusted OR(95% CI) 0.98(0.87-1.10), 0.97(0.78-1.22), and 1.00(0.83-1.19) per factor 3.3, 1.9, and 7.5 increase in exposure, respectively].

We tried to disentangle the effects of the amount of mattress dust and the mattress dust loads of the different microbial agents by first adjusting microbial agents effects for the amount of dust only, then mutually adjusting microbial agents effects, and finally by mutual adjustment with adjustment for dust in addition, i.e. by including all four exposure measures in one model. The results of the different adjustments were rather similar. Therefore, only results for the model including the four exposures are presented in Figure 3. Confidence intervals became considerably larger as a result of the correlation between the different exposures. However, the effect of the amount of dust remained statistically significant and became even slightly stronger (adjusted OR=0.57 vs 0.70). The endotoxin effect became somewhat smaller (adjusted OR=0.88 vs 0.79), and the ß(1→3)-glucan effects "disappeared" in favour of the amount of dust

effect, while there was a significant adverse effect of EPS. There was no indication of a multicollinearity problem (maximum condition index was 5.04, maximum variance inflation factor was 5.12). Mutually adjusted effects of endotoxin, ß(1→3)-glucan, and EPS loads were very similar to the effects of their concentrations presented in Figure 1, besides a somewhat stronger EPS effect.

Discussion

Our results suggest that combined across countries, higher amounts of mattress dust and higher loads of endotoxin, ß(1→3)-glucans, and EPS in mattress dust are associated with a decreased risk of allergic sensitization to inhalant allergens but not food allergens. After mutual adjustment, only the protective effect of the amount of mattress dust remained significant.

The strongest prior evidence for a protective effect of exposure to microbial agents in house dust on allergic sensitization comes from a number of studies, which consistently showed a negative association between allergic sensitization against inhalant allergens and exposure to house dust endotoxin in pre-school and schoolchildren [1-3]. No protective effect of exposure to ß(1→3)-glucan has been indicated so far. However, data on exposure to ß(1→3)-glucan on sensitization is scarce: One study reported non-significantly increased proportions of sensitized subjects among adults living in homes with higher ß(1→3)-glucan levels compared to adults living in homes with lower ß(1→3)-glucan levels [23]. In another study, no difference in prevalence of allergic sensitization was found between students attending schools with high and low ß(1→3)-

glucan levels, respectively [24]. With regard to EPS there is even less data and, to our knowledge, the association between exposure to EPS and sensitization has not been studied, yet. Likewise, there are no publications on the association between sensitization and the amount of sampled dust as a proxy for microbial exposure. Sensitization to food allergens has not been studied in any other study. Thus, comparisons of our results with the results of other studies are limited to the association between sensitization to inhalant allergens and exposure endotoxin.

The negative association between mattress dust endotoxin loads (without adjustment for the amount of dust sampled, ß(1→3)-glucan, and EPS) and allergic sensitization to inhalant allergens in the present study is qualitatively and quantitatively in line with the findings of Braun-Fahrlander et al and Gehring et al. [1;2], likewise not adjusted for the amount of dust sampled or other biocontaminants. Stronger effects for higher cut-offs for sensitization are in agreement with previous findings [2;25]. Associations between living room floor dust endotoxin and sensitization as shown by Gehring et al. [2] were not found in the present study. Our explanations for the presence of an association with mattress dust and the lack of association with living room floor dust are that the reproducibility of repeated endotoxin measurements is greater for bed dust than for floor dust [26] and that children come into closer contact with the microbial agents while sleeping. Two different ways of adjustment for amount of dust (i.e. expressing endotoxin levels per gram of dust, and including the amount of dust as an additional predictor variable in the model) yielded very similar results in the present study: the endotoxin effect became somewhat weaker. In the studies of Braun-Fahrlander et al [1] and Gehring et al. [2], however, odds ratios for endotoxin levels per gram of dust and endotoxin levels per square meter were very similar to each other. The reason for this is

not clear, but it might be explained by different correlation patterns in the different studies. Correlations between endotoxin loads and concentrations were indeed lower in the present study than in the study by Gehring et al. [2] (r = 0.60-0.81 vs r = 0.88).

When we tried to disentangle the effects of the individual exposures by mutual adjustment, only the negative association with the amount of dust remained statistically significant. The endotoxin effect became somewhat weaker while the ß(1→3)-glucan effect "disappeared". The fact that the associations between sensitization and endotoxin and ß(1→3)-glucan loads "disappear" after adjustment for the amount of dust indicate that the effects of biocontaminant loads are mainly due to the effect of the amount of dust and that there is no interactive effect of dust and biocontaminants on top of the "dust effect". However, it is not possible to distinguish with certainty, which component relates to the effect, since their levels are highly correlated with the amount of dust sampled and with each other. Therefore, we cannot rule out completely that part of the effect of the amount of dust is attributable to endotoxin ß(1→3)-glucans and EPS. Furthermore, the effect of the amount of dust could also reflect (additional) protective effects of (microbial) agents other than the ones measured.

We think that the standardized exposure assessment is one of the strengths of the present study. Associations between sensitization to inhalant allergens and the amount of dust, endotoxin, and ß(1→3)-glucans were consistent between countries – despite differences in exposure pattern. Some of the associations were not statistically significant on the country level, but they became significant when they were combined in meta-analyses. A potential limitation of the nested case-control design might be that

exposures were measured between one and four years *after* sensitization was measured. The design was chosen, because collection and analysis of house dust are time consuming and costly and therefore could not be done for the entire birth cohorts. Collection of dust before measurement of sensitization for a random sample of birth cohort members was not done due to the low prevalence of sensitization in the age group studied, which would most likely have resulted in a too small number of sensitized children resulting in too little statistical power. However, there is some evidence that a single endotoxin measurement is a valid estimate of exposure for longer time periods. [26-29] Within-home correlation was found to be considerable for living room floor dust samples over a period of 6 years (r=0.5 for endotoxin loads [29]) and somewhat higher for bed (r=0.7-0.8) [26] and bedroom floor dust samples (r=0.6) [28] over periods of up to13 months. To our knowledge, no data is available for amounts of dust, ß(1→3)-glucans and EPS. Therefore, prospective studies where exposure is measured *before* health outcomes are needed to confirm our results.

Moving to another house between blood sampling for IgE measurements and collection of house dust might result in exposure misclassification. We tried to solve this problem by excluding children who had moved between 6 months prior to blood sampling and house dust collection from the AIRALLERG study. In Germany it was not possible to strictly follow this criterion. Twenty-four percent of the children moved to another house between blood sampling and house dust collection. However, excluding these children from the analysis did not change the results (data not shown).

Assuming that a single exposure measurement is valid for a period of several years exposures measured within the AIRALLERG study could represent "current" exposures at the time (before) IgE measurements were done and just as well "early" life exposures

e.g. during the first year of life, which has been hypothesized to be crucial [30]. Exposures measured within the present study might yield a better estimate of early exposure in children who never moved to another house compared to children who moved to another home. We therefore excluded children who had moved at any time during their lives from the analysis. This did not strengthen the association between exposure to microbial agents and allergic sensitization (data not shown) indicating the relevance of current exposure over early exposure. These findings were supported by the fact that were no or low correlations between the amount of dust, endotoxin, ß(1→3)-glucans and EPS levels measured in the present study and the amount of dust, endotoxin, ß(1→3)-glucans and EPS levels on the child's mattress at age 3 months (PIAMA study, N=309 for amount of dust, N≈130 for endotoxin, ß(1→3)-glucans and EPS, maximum correlation 0.23; data from the German LISA study, N=135 for endotoxin, correlation 0.10), which is most likely due to the fact that children got new (bigger) mattresses between the two dust collections. Further evidence in favour of current exposure comes from a recent publication by Douwes et al. [11] who found no association between sensitization at four years and mattress dust microbial exposure at the age of 3 months in the PIAMA cohort.

Selective avoidance is another potential source of bias. Allergic parents for instance might tend to take measures that reduce exposure to house dust and allergens more often [19;31], and keep their houses cleaner before the baby is born. Moreover, parents of symptomatic children might respond to their children's symptoms by keeping their houses cleaner or by changing other factors that might affect exposure. Associations between endotoxin levels and cleaning habits have been shown in one study [32], but not in others [33;34] and the overall percentage of variability explained by cleaning

habits in the former study was low. The association between the amount of house dust on the child's mattress and allergic sensitization was rather similar for children with and without allergic parents [combined adjusted OR(95% CI) 0.74(0.59-0.93) and 0.61(0.43-0.86), respectively] and non-symptomatic and symptomatic children [combined adjusted OR(95% CI) 0.66(0.52-0.84) and 0.65(0.44-0.97), respectively. Thus, there is no indication of a bias due to selective avoidance measures of allergic parents or parents of symptomatic children.

The fact that the original cohorts differ with regard to inclusion criteria (GINI and PIAMA were enriched with children prone to atopy due to allergic parents), study protocols (GINI and PIAMA were at least partly designed as intervention studies), and assessment of atopy (age and set of allergens) and that selection of participants for the present study differed somewhat between countries, might be a limitation of our study. It is not possible to assess the impact of these differences on our study results. However, in spite of these differences, effect estimates were consistent between countries. Results were rather similar for children with and without allergic parents; adjustment for the presence of mattress covers and breastfeeding and exclusion of the German children who had moved between measurement of sensitization and collection of dust did not change the results. Therefore, we think that combining country-specific estimates by meta-analysis can be justified and results in valid effect estimates. Nevertheless, we cannot rule out completely that differences between study populations have an impact on our results.

In conclusion, higher amounts of mattress dust might decrease the risk of allergic sensitization to inhalant allergens. The effect might be partly attributable to endotoxin

ß(1→3)-glucans, and EPS, but could also reflect (additional) protective effects of (microbial) agents other than the ones measured. It is not possible to distinguish with certainty, which component relates to the effect, since their levels are highly correlated with the amount of dust sampled and with each other.

Acknowledgements

We wish to thank all families for their participation in the AIRALLERG study. Furthermore, we are indebted to all scientists and technical staff involved in the planning and conduction of the AIRALLERG study and the BAMSE, GINI, LISA, and PIAMA birth cohort studies. The AIRALLERG study was funded within the European Union Quality of Life and Management of Living Resources Program, Key action Environment and Health (Proposal No. QLK4-2000-00073). Ulrike Gehring was supported by a research fellowship within the Postdoc-Programme of the German Academic Exchange Service (DAAD).

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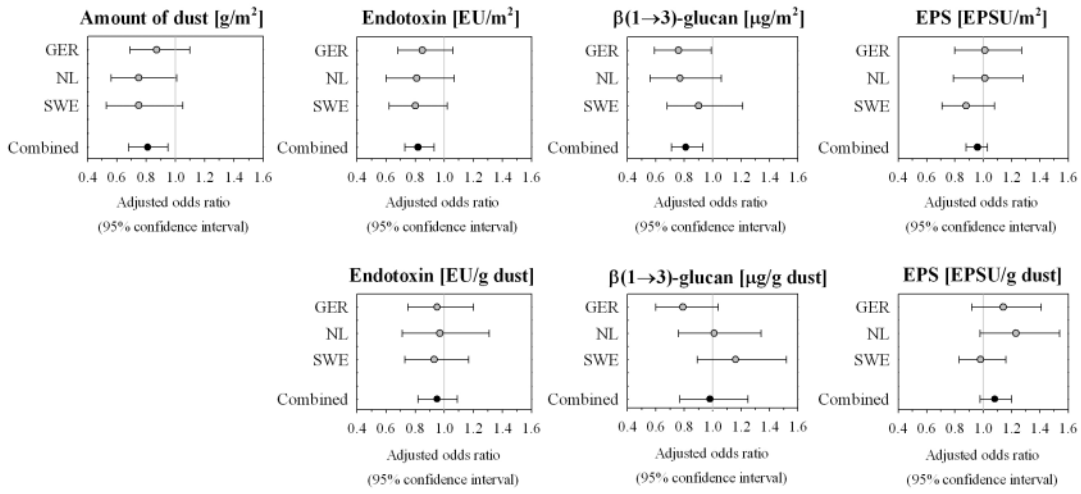
Figure legends

Figure 1. Association between allergic sensitization any allergen and amount of mattress dust sampled on the child's mattress, and endotoxin, β(1→3)-glucan, and EPS levels in the child's mattress dust. Results are presented as country-specific and combined adjusted* odds ratios (95% confidence interval) associated with a factor 2.7, 3.4, 3.4, and 5.3 increase in dust, endotoxin, ß(1→3)-glucan, and EPS exposure per m^2 , respectively.

- adjusted for sex, parental allergy, parental education and study design (natural history/intervention). Effects of biocontaminant concentrations were additionally adjusted for amount of dust sampled.



- **Figure 1**

Figure 2. Association between allergic sensitization inhalant allergen (A) and sensitization to food allergens only (B) and amount of mattress dust sampled on the child's mattress, and endotoxin, β(1→3)-glucan, and EPS per m^2 of the

child's mattress surface. Results are presented as country-specific and combined adjusted* odds ratios (95% confidence interval) associated with a factor 2.7, 3.4, 3.4, and 5.3 increase in dust, endotoxin, ß(1→3)-glucan, and EPS exposure per m^2 , respectively.

* adjusted for sex, parental allergy, parental education and study design (natural history/intervention)

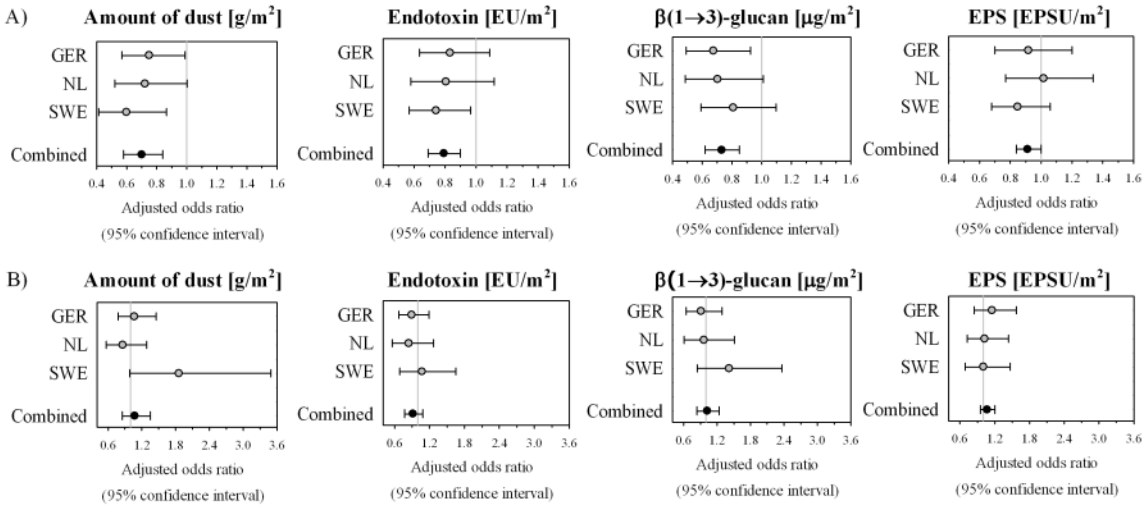


Figure 2

Figure 3. Mutually adjusted association between allergic sensitization to inhalant allergens and amount of mattress dust sampled on the child's mattress, and endotoxin, β(1→3)-glucan, and EPS levels in the child's mattress dust expressed per m^2 of mattress surface. Results are presented as country-specific and combined odds ratios (95% confidence interval) associated with a factor 2.7, 3.4, 3.4, and 5.3 increase in dust, endotoxin, ß(1→3)-glucan, and EPS exposure per m^2 , respectively, and were additionally adjusted for sex, parental allergy, parental education and study design (natural history/intervention).

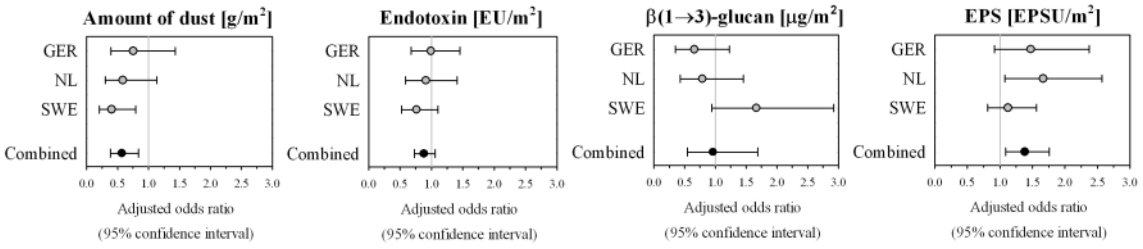


Figure 3

Table 1. Specific IgE measured in the different birth cohort studies.

	Germany		Netherlands	Sweden
	(GINI)	(LISA[¶])	(PIAMA)	(BAMSE[**])
Inhalant allergens				
Airborne allergen mix [*]				×
Animals				
Cat	×	×	×	×
Dog			×	×
Horse				×
House dust				
Dermatophagoides farinae	×	×		
Dermatophagoides pteronyssinus	×	×	×	×
House dust mix hx2 [†]		×		
Tree, grass, and weed pollens				
Birch	×		×	×
Cocksfoot			×	
Timothy grass	×			×
Tree, grass, and weed pollen mix rx1 [‡]		×		
Mugwort				×
Moulds				
Alternaria alternata			×	
Cladosporium herbarum				×
Mould mix mx1 [§]		×		
Food allergens				
Cow's milk	×	×	×	×
Egg white	×	×	×	×
Fish				×
Food mix fx 5 [‖]		×		×
Peanut				×
Soy bean	×			×
Wheat				×

[*] cat, dog, horse, birch, timothy, mugwort, *Dermatophagoides pteronyssinus*, *Cladosporium herbarum*

[†] Hollister-Stier Labs, *Dermatophagoides pteronyssinus, Dermatophagoides farinae,* German cockroach

[‡] Timothy grass, mugwort, ribwort, wall pellitory, birch

[§] *Penicillium notatum, Cladosporium herbarum, Aspergillus fumigatus, Alternaria alternata*

[‖] Egg white, milk, fish, wheat, peanut, soy bean

[¶] house dust mite specific IgE and food allergen specific IgE was measured only in children with positive house dust mix and food mix tests, respectively

** inhalant and food allergen specific IgE were measured only in children with positive airborne allergen mix and food mix, respecitvely

Table 2. Description of the study population.

	Germany (N = 356)		Netherlands (N = 338)		Sweden (N = 358)	
	n/N	**(%)**	**n/N**	**(%)**	**n/N**	**(%)**
General characteristics						
Study design [§]						
natural history	137/356	(38)	186/338	(55)	258/358	(100)
intervention	219/356	(62)	152/338	(45)	---	
Boys	203/356	(57)	191/338	(57)	183/358	(51)
Parental allergy *	297/352	(84)	265/338	(78)	72/356	(20)
High parental education	282/356	(79)	196/338	(58)	213/356	(60)
Mite-impermeable mattress cover	61/355	(17)	62/310	(20)	0/356	(0)
Pets in the child's home	90/355	(25)	140/329	(43)	111/358	(31)
Smoking in the child's home	46/356	(13)	83/330	(25)	14/357	(4)
Breastfeeding [†]	177/331	(54)	202/333	(61)	264/349	(76)
Age at blood collection in months [‡]	35 (22 – 40)		48 (46 - 55)		51 (40 – 63)	
Age at dust collection in months [‡]	63 (45 – 90)		66 (53 – 80)		87 (63 – 109)	
Never moved to another house	137/356	(38)	228/338	(67)	165/358	(46)
Allergic sensitization						
Any allergic sensitization	164/356	(46)	152/335	(45)	184/358	(51)
inhalant allergens	91/356	(26)	100/338	(30)	147/358	(41)
food allergens	109/356	(31)	89/332	(27)	104/358	(29)

* defined as asthma and/or hay fever and/or eczema (Germany), asthma and/or allergy to house dust (mite) or pets, and/or hay fever (Netherlands), asthma (Sweden)

† defined as exclusive breastfeeding during the first 4 months of life (Germany), any breastfeeding at the age of 3 months (Netherlands), and duration of breastfeeding more than 6 months (Sweden)

‡ median (minimum – maximum)

§ Germany: natural history = LISA, intervention = GINI

Table 3. Number of samples below the limit of detection, geometric mean (GM), and geometric standard deviation (GSD) of amount of dust sampled, endotoxin, ß(1→3)glucan, and EPS levels in the child's mattress dust and living room floor dust.

	Germany (N = 356)		Netherlands (N = 338)		Sweden (N = 358)	
	n<LOD*	GM (GSD)	n<LOD*	GM (GSD)	n<LOD*	GM (GSD)
Child's mattress dust						
Levels per m^2 of surface						
Amount of dust [mg/m^2]		240 (2.5)		229 (2.1)		142 (1.9)
Endotoxin [EU/m^2]	2	3053 (3.3)	0	2467 (2.5)	9	1094 (2.8)
ß(1→3)glucan [$\mu g/m^2$]	0	434 (2.8)	0	357 (2.4)	0	320 (2.5)
EPS [$EPSU/m^2$]	6	9058 (4.7)	5	7619 (4.6)	15	2173 (5.3)
Levels per gram of dust						
Endotoxin [EU/g]	2	12 515 (2.6)	0	10 763 (2.1)	9	7535 (2.5)
ß(1→3)glucan [µg/g]	0	1797 (1.7)	0	1556 (1.7)	0	2251 (1.7)
EPS [EPSU/g]	6	36 592 (3.1)	5	32 874 (3.2)	15	15 120 (4.1)
Living room floor dust						
Levels per m^2 of surface						
Amount of dust [mg/m^2]		155 (4.4)		118 (5.5)		292 (3.2)
Endotoxin [EU/m^2]	14	2874 (6.2)	23	2180 (9.7)	9	4077 (5.2)
ß(1→3)glucan [$\mu g/m^2$]	0	350 (4.9)	7	243 (7.0)	1	799 (3.7)
EPS [$EPSU/m^2$]	28	4577 (13.7)	71	1431 (30.2)	18	9699 (9.6)
Levels per gram of dust						
Endotoxin [EU/g]	14	16 837 (3.2)	23	15 124 (4.0)	9	13 122 (3.2)
ß(1→3)glucan [µg/g]	0	2225 (1.7)	7	1948 2.3)	1	2689 (1.8)
EPS [EPSU/g]	28	25 340 (6.7)	71	8700 (13.6)	18	30 641 (5.0)

* LOD = limit of detection

Table 4. Correlation between amount of dust sampled, endotoxin, ß(1→3)glucan, and EPS levels per gram of dust (upper triangle) and per m^2 (lower triangle) for the child's mattress and the living room floor.

	Child's mattress				Living room floor			
	Amount of dust	**Endotoxin**	**ß(1→3)glucan**	**EPS**	**Amount of dust**	**Endotoxin**	**ß(1→3)glucan**	**EPS**
Germany								
Amount of dust	1.00	-0.09	-0.06	0.36	1.00	0.27	0.03	0.58
Endotoxin	0.66	1.00	0.26	0.24	0.87	1.00	0.28	0.47
ß(1→3)glucan	0.85	0.67	1.00	-0.05	0.94	0.87	1.00	0.20
EPS	0.79	0.64	0.67	1.00	0.88	0.85	0.87	1.00
The Netherlands								
Amount of dust	1.00	-0.23	-0.12	0.32	1.00	0.57	0.38	0.70
Endotoxin	0.64	1.00	0.12	0.07	0.92	1.00	0.47	0.54
ß(1→3)glucan	0.81	0.56	1.00	0.05	0.96	0.92	1.00	0.32
EPS	0.72	0.55	0.63	1.00	0.88	0.85	0.86	1.00
Sweden								
Amount of dust	1.00	-0.01	0.19	0.28	1.00	0.28	0.23	0.66
Endotoxin	0.57	1.00	0.25	0.45	0.77	1.00	0.10	0.38
ß(1→3)glucan	0.82	0.59	1.00	0.19	0.92	0.72	1.00	0.28
EPS	0.60	0.64	0.56	1.00	0.87	0.75	0.85	1.00