

Asthma and the Hygiene Hypothesis

Does Cleanliness Matter?

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Abstract

Rationale: The early hygiene hypothesis explained the development of allergies by a lack of infections; nowadays, the aspect of excessive cleanliness in affluent populations seems to have replaced this concept. Yet, no investigation has shown that home or personal cleanliness relate to allergic diseases.

Objectives: To relate personal and home cleanliness to risk of asthma and allergies.

Methods: Comprehensive questionnaire information on home or personal cleanliness and allergic health conditions at school age was collected in 399 participants of the urban Perinatale Asthma Umwelt Langzeit Allergie Studie (PAULA) birth cohort. Bacterial markers were assessed in floor and mattress dust and were related to cleanliness and allergic diseases.

Measurements and Main Results: Personal cleanliness was inversely related to bacterial compounds on floors and mattresses, whereas home cleanliness effectively reduced dust amount but

not microbial markers. Exposure to muramic acid related to a lower prevalence of school-age asthma (adjusted odds ratio, 0.59 [95% confidence interval, 0.39; 0.90]). Mattress endotoxin in the first year of life was inversely associated with atopic sensitization (0.73 [0.56–0.96]) and asthma at school age (0.72 [0.55–0.95]). Despite the associations of dust parameters both with cleanliness and allergic health conditions, the development of allergies was not related to home and personal cleanliness.

Conclusions: Bacterial exposure in house dust determined childhood asthma and allergies. Personal cleanliness, such as washing hands, and home cleanliness were objectively reflected by dust parameters in homes. However, neither personal nor home cleanliness was associated with a risk for asthma and allergies. Other microbial components in house dust not affected by personal hygiene are likely to play a role.

Keywords: endotoxin; muramic acid; childhood; house dust; allergies

The hygiene hypothesis is widely accepted in science as well as in the public domain to explain the rapid rise in allergic disorders in affluent populations by an increase in hygiene. Originally conceived as a lack of infections predisposing to allergies, the hygiene hypothesis is sometimes reduced to formulas such as “eat dirt” (1), “too clean is

bad,” or “sterile homes” (2), although clean homes are far from being sterile.

In a scientific context, “excessive cleanliness” has been implied in reduced “microbial stimulation needed to enable normal development of the immune system” (3). To a lay audience, the U.S. Food and Drug Administration explains the

hygiene hypothesis by “the extremely clean household environments often found in the developed world” (4).

Yet, the question remains whether the insinuated association between cleanliness and development of asthma and allergies can be substantiated in human populations. Certainly, strong correlations of microbial

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At a Glance Commentary

Scientific Knowledge on the

Subject: The increase of asthma and allergies has been linked to the improvement of hygiene standards in affluent populations. However, it has never been shown that personal hygiene and domestic cleanliness relate to asthma and allergic diseases.

What This Study Adds to the

Field: Development of allergies and asthma was not related to cleaning activities.

compounds in the home environment and childhood asthma, wheeze, and atopic sensitization have been found repeatedly in epidemiological studies (5–7). In the context of studies on the composition of house dust, cleaning habits such as sweeping the floor at the sampling site or changing the bedding have been assessed (8–11). However, the ties between household or personal cleanliness and development of allergies have never been established.

Hence, we sought to test this tacit assumption in an urban birth cohort by collecting comprehensive information on home and personal cleanliness, measuring bacterial markers in indoor dust, and evaluating health conditions such as asthma and allergic diseases. We first assessed the relation of indoor dust markers to their known determinants, such as keeping pets or number of children. Then we validated our cleanliness variables against the objectively measured indoor dust markers and confirmed protective effects of dust markers on asthma and allergic conditions. Finally, we tested the supposed association of home and personal cleanliness with asthma and allergic conditions.

Some of the results of these studies have been previously reported in the form of an e-poster (12).

Methods

The Cohort

The Perinatale Asthma Umwelt Langzeit Allergie Studie (PAULA) birth cohort has been set up to study the effect of lifestyle and environmental factors on the development of asthma and allergy. Initially 553 families, including 40 pilot families, were recruited in

the urban and suburban regions of Munich, Germany, between October 1999 and December 2001. The children have been followed up on a yearly basis until age 5 years (13). At age 8 to 11 years we contacted all families who participated in the 5-year follow-up. The study was approved by the local ethics committee.

Questionnaires

We addressed the issue of cleanliness in the household and of personal cleanliness during the follow-up at 8 to 11 years by a multitude of items listed in Table 1. The telephone interview comprised 31 questions to comprehensively assess cleaning habits

and cleaning frequencies in four to five categories in the homes, the use of detergents, and the personal cleanliness of the study child (see Figure E1 in the online supplement). In addition, we assessed 13 items of lifestyle factors and home characteristics by a self-administered questionnaire along with the dust collection kit.

Questions about the child's health focused on respiratory and allergic problems and their treatment. The data were collected by telephone interview; the information gap from age 5 years to the most recent study at age 8 to 11 years was closed by assessing cumulative prevalences since the last follow-up. Asthma was defined as reported

Table 1. List of Variables Related to Personal and Home Cleanliness and Home Characteristics

Home cleanliness

Frequency of

- ... cleaning the soother by boiling in the child's first year*
- ... sweeping, vacuuming, or wiping floors*
- ... wiping dust[†]
- ... dry cleaning or shampooing rugs[†]
- ... vacuuming mattresses[†]
- ... cleaning the toilet/kitchen/bathroom*
- ... cleaning windows*
- ... changing towels in kitchen, toilet, and bathroom*
- ... use of washing powder, liquid detergents for floors, liquid detergents for smooth surfaces, bleach, ammoniac, decalcifier, organic solvents, toilet cleaner[†]

Habit of

- ... taking off shoes before entering the home*
- ... sharing towels in kitchen, toilet, and bathroom[†]

Child's personal cleanliness

Frequency of

- ... washing hands after playing outdoors/before meals/after using the toilet/with soap*
- ... cutting fingernails/toenails[†]
- ... taking a bath, taking a shower[†]
- ... washing hair with shampoo*
- ... changing toothbrush[†]
- ... changing bed sheets*
- ... changing underwear*
- ... swimming in an indoor/outdoor or natural pool[†]

Habit of

- ... sleeping in clothes from the day*
- ... chewing finger nails (yes, no)
- ... washing feet before bedtime after walking with bare feet[†]

Home characteristics and lifestyle

- Number of family members, children, visitors, smokers in the home
- Level of child's room (first floor or lower, above first floor)
- Level of family room (ground floor or lower, above ground floor)
- Pets (which, how many, where, in bed?)
- Guest animals[§] (yes/no)
- Ventilation (five questions)
- Indoor plants (yes/no)
- Moisture damage, mold and damp spots, humidifier, fish tank (yes/no)
- Garden, front yard (yes/no)
- Compost container indoors/outdoors (yes/no)

*Four categories.

[†]Five categories.

[‡]Three categories.

[§]Animals that are brought into the home by visitors or are under care at the home during limited periods.

doctor-diagnosed asthma ever or more than one episode of asthmatic or obstructive bronchitis ever. The case definition for atopic dermatitis was a reported doctor diagnosis ever. Hay fever was considered present if the child was sensitized to any aeroallergen (assessed by skin prick test) and ever had sneezing or a runny, blocked, or itchy nose together with runny or itchy eyes without having a cold after the third birthday.

Skin Prick Test

Atopic sensitization was measured by skin prick test positivity at age 5 years. A child was defined as sensitized to an allergen (*Dermatophagoides pteronyssinus*, *Dermatophagoides farinae*, cat, *Alternaria*, grass mix, birch, and hazelnut) if it provoked a mean wheal diameter at least 2 mm greater as compared with saline control.

Dust Collection

Dust samples from family room floors and from mattresses were collected as part of a home visit when the child was 3 months old (13). Follow-up dust sampling at age 8 to 11 years was conducted throughout the 2010 calendar year; however, 95% of all samples were collected between March and October. The participating families sampled dust from the family room floor and from the child's mattress with their own vacuum cleaners by the sock method described by Schram-Bijkerk and colleagues (14). In homes with mainly smooth floor, 4 m² were vacuumed for 4 minutes; on carpeted floors, 1 m² was vacuumed for 2 minutes. Mattress dust was obtained by vacuuming all layers of the bedding simultaneously for 2 minutes after removing the pillow and the blanket. Before weighing the dust sample, large compounds such as hair, bread crumbs, or wooden pieces were removed.

Microbial Marker Assays

Endotoxin content of dust samples was determined by a kinetic limulus amoebocyte lysate assay (QCL; Lonza Inc., Walkersville, MD; LOT 76367, potency against *Escherichia coli* O55:B5: 13 EU/ng) as described earlier (15). Briefly, dust samples were extracted with endotoxin-free water (dilution 1:700), and an aliquot of 100 µL suspension was added to a microtiter plate and analyzed. Duplicates of each sample were spiked with an endotoxin standard (recoveries 75–120%). A standard calibration curve (0.05–0.5–5–50 EU/mL), a laboratory blank, and an internal laboratory

standard were included on each plate. Endotoxin results were expressed as units per milligram of dust (concentration) and as units per square meter of surface area of the sampled location (load). Muramic acid levels were measured by combined gas–liquid chromatography/mass spectrometry as described before (6). Muramic acid results were reported as nanograms per milligram of dust (concentration) or nanograms per square meter of vacuumed area (load). When dust quantities were low, endotoxin measurements took priority over muramic acid measurements; hence, muramic acid was only measured in 244 mattress and 218 floor samples.

Statistical Analysis

Data analysis was performed with the SAS 9.2 software (The SAS Institute, Cary, NC). *P* values less than 0.05 were considered statistically significant. For a sensitive selection of potential confounders, a more progressive significance level was applied ($P < 0.1$). Bacterial markers and dust weights were analyzed as *z*-transformed, continuous variables. *z*-Scores for floor dust samples were created separately for carpeted and smooth floors, because the latter yielded substantially less dust. Cleanliness variables

were used in four or five categories. For home cleanliness 24 variables and for the child's personal cleanliness 17 variables were combined into two scores (Table 1). Two questionnaire variables ("dry cleaning or shampooing rugs" and "swimming in a natural pool in summer") were not considered for the cleanliness scores, because they had more than 10% missing values. All other variables had few missing values (on average 2%) and were imputed by multiple imputation resulting in 10 randomly generated datasets. To account for mutual correlations among the cleanliness variables within a score, we modeled the respective contribution of each variable by factor analysis in each imputed dataset. The cleanliness scores represent the factors explaining the majority of variance among the respective variables and were derived by averaging over the 10 imputed datasets. The resulting scores were categorized into quintiles to account for skewed distributions. Correlations were determined by Spearman ρ . Logistic and linear regression models were used to calculate associations between allergy outcomes, dust exposures, and cleanliness scores. All models were adjusted for sex and maternal education.

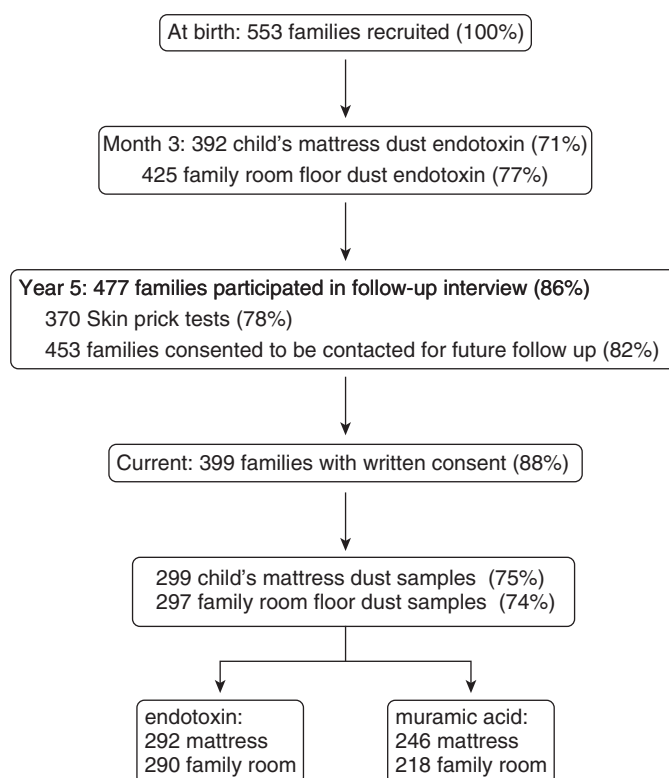


Figure 1. Selection of study population. Percentages refer to the next higher level.

Table 2. Crude Associations of Floor Dust Parameters with Lifestyle and Home Characteristics

Variable	Dust Weight			Endotoxin Load			Endotoxin Concentration			Muramic Acid Load			Muramic Acid Concentration		
	β	[95% CI]	P Value	β	[95% CI]	P Value	β	[95% CI]	P Value	β	[95% CI]	P Value	β	[95% CI]	P Value
Female sex	-0.058	[-0.288; 0.172]	0.62	-0.180	[-0.409; 0.049]	0.12	-0.255	[-0.483; -0.027]	0.03	-0.188	[-0.452; 0.075]	0.16	-0.257	[-0.520; 0.005]	0.055
Mother has university degree	0.126	[-0.103; 0.356]	0.28	0.191	[-0.038; 0.420]	0.10	0.185	[-0.044; 0.414]	0.11	0.014	[-0.251; 0.280]	0.92	-0.007	[-0.272; 0.259]	0.96
More than two children in home	0.368	[0.087; 0.648]	0.01	0.295	[0.012; 0.577]	0.04	0.095	[-0.190; 0.381]	0.51	0.536	[0.227; 0.845]	0.007	0.261	[-0.055; 0.577]	0.11
More than four visitors/wk	0.113	[-0.125; 0.350]	0.35	0.282	[0.047; 0.518]	0.02	0.271	[0.035; 0.506]	0.03	0.196	[-0.077; 0.469]	0.16	0.160	[-0.113; 0.434]	0.25
Pets	0.111	[-0.115; 0.337]	0.34	0.222	[-0.010; 0.455]	0.06	0.185	[-0.048; 0.417]	0.12	0.239	[-0.026; 0.505]	0.08	0.095	[-0.173; 0.363]	0.49
Guest animals*	-0.057	[-0.346; 0.232]	0.70	0.274	[-0.015; 0.563]	0.06	0.505	[0.220; 0.791]	0.0005	-0.011	[-0.342; 0.321]	0.95	0.132	[-0.201; 0.465]	0.44
House with garden†	0.057	[-0.300; 0.415]	0.75	0.292	[-0.064; 0.647]	0.11	0.515	[0.164; 0.867]	0.004	0.157	[-0.291; 0.604]	0.49	0.139	[-0.307; 0.585]	0.54
Level of family room on ground floor or lower†	0.106	[-0.164; 0.376]	0.44	0.266	[0.001; 0.531]	0.05	0.350	[0.081; 0.618]	0.01	0.317	[-0.001; 0.636]	0.05	0.359	[0.044; 0.675]	0.03
Level of child's room on first floor or lower†	-0.015	[-0.308; 0.277]	0.92	0.185	[-0.107; 0.476]	0.22	0.361	[0.071; 0.650]	0.02	0.242	[-0.105; 0.589]	0.17	0.272	[-0.074; 0.618]	0.12

Definition of abbreviations: β = beta estimates; CI = confidence interval.

z-Transformed dust parameters were regressed on cleanliness scores, which were coded in quintiles. β -Estimates reflect increase of units of SD per quintile of the cleanliness scores.

*Animals that are brought into the home by visitors or are under care at the home during limited periods.

†Variables assessed in subjects participating in the dust collection (N = 292).

Table 3. Crude Associations of Mattress Dust Parameters with Lifestyle and Home Characteristics

Variable	Dust Weight			Endotoxin Load			Endotoxin Concentration			Muramic Acid Load			Muramic Acid Concentration		
	β	[95% CI]	P Value	β	[95% CI]	P Value	β	[95% CI]	P Value	β	[95% CI]	P Value	β	[95% CI]	P Value
Female sex	0.073	[-0.156; 0.302]	0.53	-0.074	[-0.303; 0.155]	0.53	-0.196	[-0.424; 0.032]	0.09	-0.047	[-0.297; 0.203]	0.71	-0.049	[-0.299; 0.201]	0.70
Pets	0.188	[-0.040; 0.416]	0.11	0.299	[0.070; 0.527]	0.01	0.298	[0.068; 0.528]	0.01	0.308	[0.059; 0.557]	0.02	0.189	[-0.065; 0.442]	0.14
Guest animals*	-0.160	[-0.446; 0.127]	0.28	0.044	[-0.244; 0.332]	0.76	0.332	[0.045; 0.619]	0.02	-0.163	[-0.480; 0.154]	0.31	0.018	[-0.300; 0.335]	0.91
Dust sampling in cold season†	-0.174	[-0.436; 0.089]	0.20	-0.244	[-0.506; 0.017]	0.07	-0.157	[-0.419; 0.105]	0.24	0.243	[-0.049; 0.536]	0.10	0.441	[0.152; 0.730]	0.003
Using dryer to dry laundry†	-0.280	[-0.511; -0.049]	0.02	-0.317	[-0.548; -0.086]	0.007	-0.207	[-0.439; 0.026]	0.08	-0.226	[-0.481; 0.028]	0.08	-0.051	[-0.307; 0.206]	0.70
Mold spots in the home in the last 12 mo†	0.085	[-0.195; 0.365]	0.55	0.122	[-0.159; 0.404]	0.39	0.122	[-0.157; 0.402]	0.39	0.345	[0.033; 0.657]	0.03	0.128	[-0.186; 0.441]	0.43

Definition of abbreviations: β = beta estimates; CI = confidence interval.

z-Transformed dust parameters were regressed on cleanliness scores, which were coded in quintiles. β -Estimates reflect increase of units of SD per quintile of the cleanliness scores.

*Animals that are brought into the home by visitors or are under care at the home during limited periods.

†Variables assessed in subjects participating in the dust collection (N = 292).

Results

Study Population

The selection of the study population is given by Figure 1. Participation in the current follow-up was significantly higher with an individual or family history of allergy or a high maternal education (data not shown). Families with smokers in their homes were significantly less likely to participate (odds ratio, 0.56 [95% confidence interval, 0.44; 0.96]). The subsample of children with complete values for dust marker measurements was slightly enriched for atopic children (Table E1). The children of the study population were between 7.8 and 11.0 years old (mean, 9.4 yr). Asthma, hay fever, and atopic sensitization were significantly less common in girls (Table E2).

Determinants of Indoor Dust Markers

Unlike muramic acid levels, endotoxin levels were much higher in the floor than in the mattress samples (Table E3). The correlation of endotoxin concentrations in floor dust between early infancy and school age was substantial (Pearson $r = 0.58$). Dust markers in family room floors were significantly associated with sex, sibling frequency, pets or guest animals, level of the sampled room, number of weekly visitors, or if the home had a garden attached (Table 2). Mattress dust parameters were significantly associated with pets and guest animals, using a dryer to dry laundry, and mold spots in the home (Table 3).

Cleanliness Scores and Their Relation to Dust Parameters

The two cleanliness scores were moderately correlated ($\rho = 0.31$). The score for home cleanliness was most strongly correlated with “changing towels,” “wiping dust,” “cleaning the floor,” and “cleaning the bathroom” (Table E4). Home cleanliness was inversely related to high maternal education and positively to number of visitors, presence of pets, or guest animals (Table E6). The score for personal cleanliness correlated most strongly with “washing hands,” “washing feet,” “taking a shower,” and “cutting toenails” (Table E5) and was positively associated with female sex (Table E6).

The home cleanliness score was inversely related to dust amount in the family room, whereas personal cleanliness was inversely associated with dust amount and endotoxin load, but not concentration,

in floor and mattress samples (Figure 2). In addition, an inverse relation of personal cleanliness with mattress endotoxin at 3 months was observed ($P = 0.07$).

Associations of Dust Parameters and Health Conditions

An asthma diagnosis was inversely related to high muramic acid load in the family room at school age and to endotoxin concentration in mattress dust collected in infancy (Figure 3; see Model 1 in Table E7). The latter exposure also reduced the risk of atopic sensitization. Atopic eczema was inversely related to dust amount, endotoxin load, and muramic acid load in mattress dust. Similarly, risk of hay fever decreased with exposure to dust amount and endotoxin load in mattress dust.

Effect of Cleanliness on Health Conditions

Because dust parameters were both associated with cleanliness scores (Figure 2) and health conditions (Figure 3), we investigated the immediate relation of cleanliness scores and health conditions. However, there were no significant associations (Figure 4) also when adjusting for microbial markers (Table E8). Likewise, the effects of dust parameters on the health conditions were not changed by the scores (Table E7). As summarized by Figure 5, cleanliness and health outcomes were independently of each other associated with dust parameters. A more detailed synopsis of these associations is provided in Figure E2.

Where applicable, models were tested for confounding by the variables number of siblings, family history of allergy, pet keeping, house with garden, level of the sampled room, using a dryer, water damage ever, sampling season, or indoor mold in the last 12 months. However, none of the above variables substantially changed the estimates of the investigated associations (data not shown), and, consequently, they were not considered in the final models. Mold in the home was a strong independent risk factor for asthma (adjusted odds ratio, 2.93 [95% confidence interval, 1.11; 7.69]).

Discussion

The hygiene hypothesis underwent several transformations since its early origins when, for example, Gerrard and colleagues proposed in 1976 that “relative freedom from

infectious diseases in childhood . . . might lead to an increase in the prevalence of atopic disease” (16) or when David Strachan hypothesized in 1989 that “allergic diseases were prevented by infection in early childhood, transmitted by unhygienic contact with older siblings” (17). At that time the relevance of early manifest infections was the primary focus of research. Since then, the increasing use of disinfectants in home environments shifted the focus from manifest infections toward “sterile” homes and may have paved the way toward a kind of “cleanliness hypothesis.”

Because of its relevance for prevention strategies, we were interested whether this redraft of the hygiene hypothesis could be substantiated by observational data. Hence, we evaluated the mutual associations between cleanliness, dust exposure, and health conditions (Figure 5). We investigated cleaning behavior and individual cleaning habits by a comprehensive questionnaire covering more than 40 relevant items. When confronted with the problem of how to operationalize home and personal cleanliness, we noticed considerable heterogeneity within our urban birth cohort for many variables but little variability in the overall cleanliness patterns. In our opinion, the most appropriate approach was to include all available information in two distinct measures possibly reflecting microbial contamination encountered on indoor surfaces and microorganisms colonizing body habitats. Obviously, there is no gold standard for modeling such an abstract concept as cleanliness. Our approach, however, corresponded to objective dust measurements (Figure 2), thereby demonstrating its usefulness. Retrospectively, the differential assessment of personal and home cleanliness was justified by their relatively low correlation and their distinct associations with dust parameters.

Personal cleanliness was inversely associated with dust quantity and endotoxin burden in mattress samples, which plausibly reflect microbial exposure on a personal level. Interestingly, our model of personal cleanliness correlated most strongly with washing hands regularly (i.e., a rather effective way of reducing the spread of infectious agents) (18, 19). The unexpected but consistent associations of personal cleanliness with parameters in floor dust from family rooms, however, may point to a link between children’s behavior and

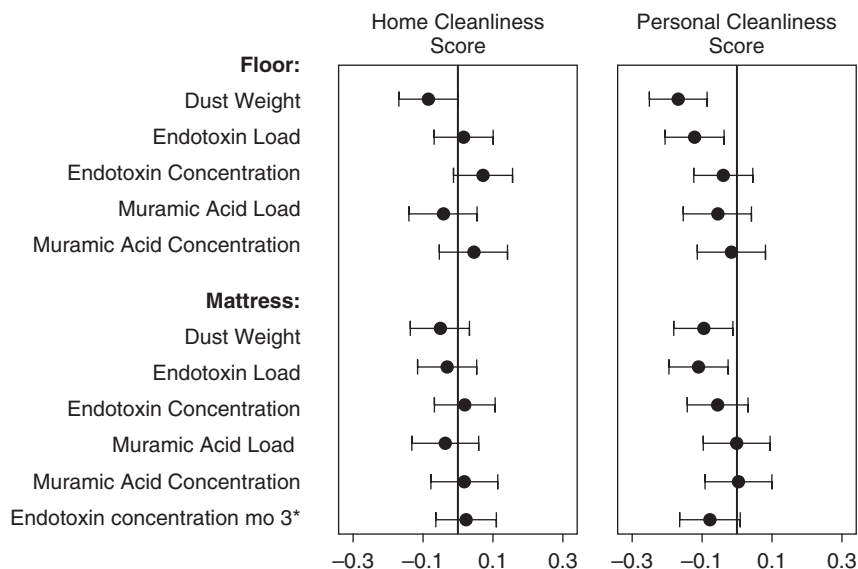


Figure 2. Mutually adjusted associations of cleanliness scores with dust parameters. z-Transformed dust parameters were regressed on cleanliness scores, which were coded in quintiles. β -Estimates reflect increase of units of SD per quintile of the cleanliness scores. β -Estimates are adjusted for sex and maternal education and mutually for both cleanliness scores. *Dust sample collection at age 3 months.

family habits, which are determined by parental role models (20) and are rather stable over time (21).

Home cleanliness resulted only in quantitative reduction of floor dust, which mainly indicates removal of superficial dirt with a rather cosmetic effect. Conventional cleaning does not eradicate microorganisms sustainably, because emptied microbial niches are instantly recolonized by ventilation and living carriers (22). The entire home community, including visitors and pets, are major vehicles of transport and deposition of microbial compounds. In this context, causation might be reverse;

that is, stronger indoor contamination by more children, visitors, animals, or proximity to a garden may prompt more intensive cleaning (Table E6), yet without any impact on bacterial markers (Figure 2). Moreover, vigorous household cleaning may lead to dispersion of dust microbes and fungal elements and paradoxically increase airborne dust markers but not necessarily dust reservoirs, which our measurements represent.

The next link in the supposed causal chain from cleanliness toward asthma and allergies consists in the associations of dust parameters and disease status. So far, the

pertinent studies have produced partially conflicting results (8, 9, 23, 24), which might be explained by different study settings or geographical variation. In the present study population, however, there were clear inverse associations of endotoxin concentration in the first year of life with asthma and atopic sensitization, mattress dust weight and endotoxin load with eczema and hay fever, mattress muramic acid with eczema, and floor muramic acid with asthma (Figure 3).

Because we found inverse associations of cleanliness with the dust parameters and inverse associations of dust parameters with asthma and allergies, we expected positive associations of cleanliness with the disease outcomes. The expectation, however, was not met (Figure 5). This is the central finding of the present analysis and demonstrates that the validity of the hygiene hypothesis is independent of cleanliness. If the parameters we measured in indoor dust do not link cleanliness and disease, once more the question arises what dust parameters ultimately stand for. Indeed they may be suitable measures for the overall microbial burden as demonstrated by the strong associations with household-related determinants such as garden proximity, pets, and number of children. The association of dust parameters with the disease outcomes, however, might reflect more specific microbial exposures, which may be detectable in some settings but suppressed by a low signal-to-noise ratio in other contexts. Endotoxin, for example, has been shown to reflect a rather unspecific, collective signal for diverse bacterial

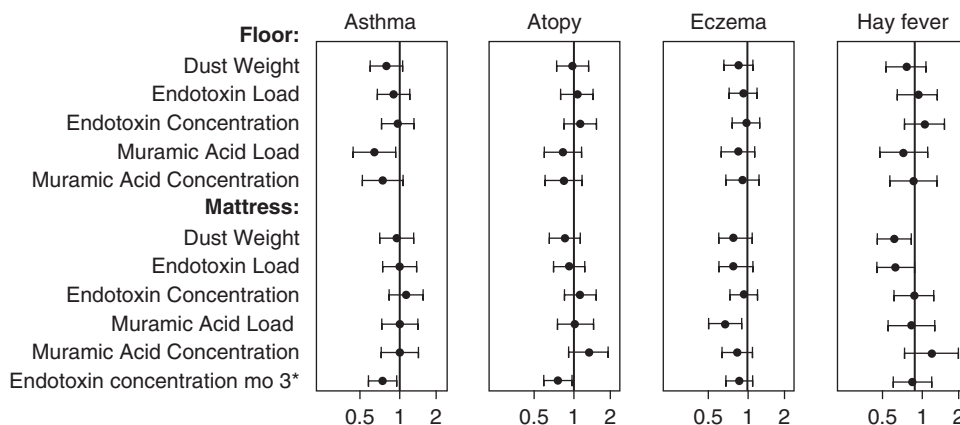


Figure 3. Associations of dust parameters with asthma, atopic eczema, hay fever, and atopic sensitization. Atopy was measured by skin prick test at age 5 years. Odds ratios refer to units of SD of z-transformed dust parameters. Each model was adjusted for sex and maternal education but not for the other dust parameters. *Dust sample collection at age 3 months.

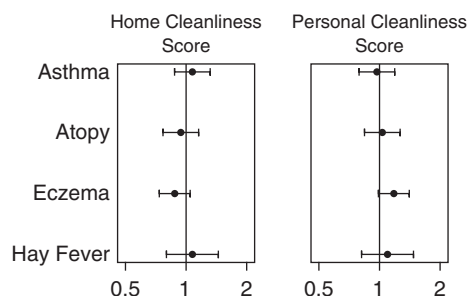


Figure 4. Mutually adjusted associations of cleanliness scores with asthma, atopic sensitization, atopic eczema, and hay fever. Atopy was measured by skin prick test at age 5 years. Each model was adjusted for sex and maternal education but not for the other dust parameters. Odds ratios are adjusted for sex and maternal education and mutually for both cleanliness scores. Odds ratios refer to cleanliness score quintiles.

exposures, among them protective and risk effects, which prevail in varying shares or may even cancel out (25). In many studies, endotoxin is the only marker of bacterial exposure and not adjusted for potential microbial confounders, which might convey the antiallergic effect instead. Obviously, endotoxin is a marker of fecal contamination; in a mouse model of colonization with human fecal bacteria, however, only clusters of the gram-positive clostridia induced T-regulatory cells and production of IL-10, two key factors in allergy inhibition (26).

Whether specific microbial communities, the balance between microorganisms, or their diversity eventually leads to development or prevention of asthma and allergies is still to be elucidated (27, 28).

A major strength of the present analysis is the unique opportunity to study the triangle of cleanliness, dust markers, and health outcomes in a birth cohort. The effects of pets, crowding, and garden proximity on indoor endotoxin (9, 10); the

protective effect of early life endotoxin exposure on asthma risk at school age (5, 8); and the increase of asthma risk by visible mold spots (29–31) are in line with previous reports and support the validity of our findings.

A limitation might be seen in measuring cleanliness, dust markers, and health outcomes only on one or two occasions and not in a time sequence clearly separating exposures and outcomes. However, our measurements are likely to have captured these continuous conditions adequately for two reasons. First, current cleanliness is a good indicator for past behavior (21), and children unescapably copy their parents' habits (20). This notion is also supported by the strong correlation of personal cleanliness, as ascertained by a single interview at 10 years, with mattress dust endotoxin levels measured already at 3 months of age. Second, dust markers themselves proved stable over time in our cohort, as illustrated by the substantial correlation of endotoxin concentrations in floor dust samples collected 10 years apart.

A further shortcoming is that 25% of children did not provide dust samples. However, the restricted dust marker dataset was sufficiently powered to detect significant associations of dust markers with both cleanliness and health conditions. In contrast, we failed to detect a link between cleanliness and allergy in the full data set with even higher power.

Additionally, generalizability might be limited, because our study participants were drawn from an affluent (sub)urban population.

Taken together, the current results suggest that neither home nor personal cleanliness impact on the development of asthma and allergies, although cleanliness was substantiated by objective, but rather unspecific, dust measurements. These quantifiable dust exposures indeed protected from asthma and allergies in our

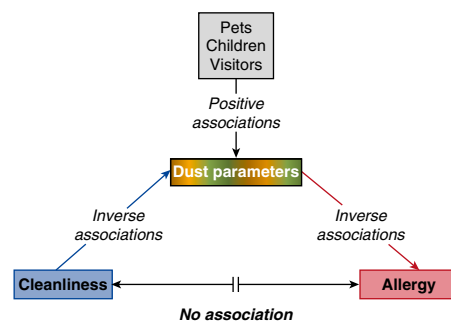


Figure 5. Summary of assessed associations.

prospective study. These findings suggest that allergy protection operates through definite yet unknown microbial exposures in homes, which cannot accurately be assessed by unspecific markers such as endotoxin or muramic acid. Future studies will require more profound microbial exposure analyses capturing identification of distinct species and functional aspects. Whether these functional aspects are affected by conventional cleaning activities remains to be elucidated; our findings with unspecific markers, however, suggest that normal cleaning does not affect permanent microbial colonization of indoor environments. Recontamination by hands, skin, or textiles leaves conventional cleaning a Sisyphean labor and dismisses the idea of “excessive cleanliness” and “sterile homes” as a cause of allergy. ■

Author disclosures are available with the text of this article at www.atsjournals.org.

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