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House dust endotoxin, asthma and allergic sensitization through childhood into adolescence

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Abstract

Background: House dust endotoxin may have beneficial effects on allergic sensitization and asthma in children. Evidence is scarce for adolescents and most studies so far have been cross-sectional and limited to a single exposure measurement.

Objective: We assessed associations of house dust endotoxin with asthma and allergic sensitization from birth to age 17 years longitudinally taking into account exposure early in life and at primary school age.

Methods: We used data of 854 participants of the prospective Dutch PIAMA birth cohort study with house dust endotoxin measurements at 3 months and/or 5-6 years and data on asthma and/or allergic sensitization from at least one of 11 follow-ups until age 17. We assessed overall and age-specific associations of the prevalence of asthma and sensitization with mattress and living room floor dust concentrations (per gram of dust) and loads (per m² of sampling surface).

Results: Higher living room floor dust endotoxin concentrations at 3 months were associated with lower odds of asthma until age 4 [odds ratio (95% confidence interval) ranging from 0.70 (0.51-0.97) at age 1 to 0.76 (0.57-1.00) at age 3 per interquartile range increase], but not thereafter in children of allergic mothers. Higher living room floor dust endotoxin at 5-6 years was associated with higher odds of sensitization at 8-16 years [eg odds ratio (95% confidence interval) 1.70 (1.17-2.47) per interquartile range increase in endotoxin load].

Conclusions and clinical relevance: House dust endotoxin may have beneficial effects on asthma in preschool children of allergic mothers, which do not persist into adolescence. Beneficial associations with allergic sensitization could not be confirmed.

KEYWORDS

allergic sensitization, asthma, birth cohort, endotoxin

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1 | INTRODUCTION

Asthma is one of the most common chronic diseases in childhood. It has been estimated to affect some 235 million people worldwide.¹ The causes of asthma are not completely understood, but both genetic and environmental factors have been shown to be involved.^{2,3}

Endotoxin (lipopolysaccharide [LPS]), a constituent of the outer membrane of gram-negative bacteria, has gained interest as a determinant of asthma and allergy during the past decades due to its strong immune-stimulatory and pro-inflammatory properties. Several studies reported lower risks of allergic sensitization with higher exposure to house dust endotoxin in children and young adults living in rural and urban environments.⁴⁻¹¹ Findings of studies assessing associations of house dust endotoxin with asthma and related symptoms are less consistent.^{12,13} Higher levels of endotoxin have been reported to be associated with both increased^{15,14-16} and decreased^{4,10,17-20} risks of asthma (symptoms) in children and adolescents. A meta-analysis of nineteen studies of the associations of house dust endotoxin with asthma and wheeze concluded that early life exposure to endotoxin is associated with an increased risk of wheeze in infants and toddlers (odds ratio [95% confidence interval] 1.48 [1.10-1.98]), while current exposure to endotoxin is associated with a decreased risk of asthma in schoolchildren (0.68 [0.50-0.93]).¹³ This suggests that associations of asthma with endotoxin are age-dependent and/or that exposure during different time periods has differential effects. The relevance of the timing of exposure in addition to the level has also been indicated in animal experiments.²¹ A limitation of many previous studies, including the majority of studies that were performed within prospective cohorts, is the cross-sectional design and the lack of repeated exposure measurements, which makes it impossible to disentangle the relevance of the timing of exposure from the relevance of age.

Therefore, we assessed associations of house dust endotoxin with asthma and allergic sensitization from birth to age 17 years longitudinally taking into account exposure early in life and at primary school age. The mechanisms underlying such associations are not entirely clear, but endotoxin has strong immune-stimulatory and pro-inflammatory properties through which it can promote asthma by inducing airway inflammation on the one hand and may have protective effects against atopy development by activating type 1 T helper cell responses on the other hand.²² Endotoxin interacts with CD14-positive cells such as monocytes and macrophages via the CD14 receptor and with CD14-negative cells such as endothelial and epithelial cells through a soluble form of CD14.^{23,24} Since it has been suggested that Toll-like receptor (TLR) 4 mutations are associated with endotoxin hyperresponsiveness²⁵ and the association between endotoxin and allergic sensitization has been found to be modified by single-nucleotide polymorphisms (SNPs) in the CD14 receptor,^{26,27} we also assessed potential modifications of associations with endotoxin by a common SNP in the CD14 receptor (rs2569190) and SNPs in the TLR4 for which effect modifications with endotoxin have been assessed previously.²⁸

2 | METHODS

2.1 | Study design and study population

Details on the PIAMA birth cohort study have been published elsewhere.^{29,30} In brief, pregnant women were recruited from the general population in the north, west and centre of the Netherlands in 1996-1997 through antenatal clinics. Non-allergic pregnant women were invited to participate in a "natural history" study arm. Pregnant women identified as allergic through the screening questionnaire were allocated primarily to an intervention arm with a random subset allocated to the natural history arm. The intervention involved the use of mite-impermeable mattress and pillow covers, but had no effect on the development of asthma and allergy³¹ and therefore, participants of both the intervention and the natural history study were included in the present study.

The study started with 3963 newborns. Parents completed questionnaires on demographic factors, risk factors for asthma and respiratory symptoms at birth, at the child's ages 3 of months and 1 year and then annually until the age of 8 years. At ages 11, 14 and 17 years, both the parents and the participants themselves completed questionnaires. Measurements of specific immunoglobulin E (IgE) were performed at ages 4, 8, 12 and 16 in subpopulations with an over-representation of children of allergic mothers at age 4. House dust samples were collected during home visits for subsets of the population with an over-representation of children of allergic mothers during pregnancy when the children were 3 months, 1 and 5-6 years old. Subsets with house dust endotoxin levels during pregnancy and at age 1 year were too small for reliable epidemiological analyses. Therefore, we restricted the current study to participants with questionnaire data on asthma at any age from age 1 to 17 years and house dust endotoxin levels at 3 months and/or 5-6 years ($n = 857$, see Figure S1 for more details).

The Institutional Review Boards of the participating institutes approved the study protocol, and written informed consent was obtained from the parents or legal guardians of all participants.

2.2 | Health outcomes

Asthma was defined as a positive answer to at least two of the three following questions: (a) "Has a doctor ever diagnosed asthma in your child?," (b) "Has your child had wheezing or whistling in the chest in the last 12 months?," (c) "Has your child been prescribed asthma medication during the last 12 months?," a definition that has been developed by a panel of experts within the MeDALL consortium.³² Those with two or more negative answers to the three questions were considered non-asthmatic. Measurements of specific IgE levels against common aero allergens were performed in blood samples collected at 1, 4, 8, 12 and 16 years (Table S1). Allergic sensitization to any (inhalant and food) allergens was defined as a specific IgE level of ≥ 0.35 IU/mL to one or more of the (inhalant and food) allergens tested.

2.3 | House dust collection and endotoxin measurements

House dust samples have been collected on the child's mattress and the living room floors in homes of children of allergic mothers during pregnancy and the child's first year of life and sensitized and non-sensitized children of predominantly allergic mothers at age 5-6 years as described previously^{33,34} and in the online supplement. Dust including filters was weighted and extracted as described elsewhere³⁵ and extracts were stored frozen until analysis. Endotoxin was measured in extracts by a chromogenic kinetic Limulus amoebocyte lysate test³⁶ and endotoxin levels were expressed per gram of sampled dust (as a measure of concentration) and per m² of sampling surface area (as a measure of the total load/burden).

2.4 | Genotyping

Genotyping was done using three different platforms: Illumina Omni Express Exome (OEE) chip (n = 1377), Illumina Omni Express (OE) chip (n = 288) and Illumina Human610 (HM610) quad array (n = 404) (Illumina Inc, San Diego, CA). The *CD14/rs2569190*, *TLR4/rs2737190* (in complete linkage disequilibrium with *TRL4/rs1927914*), *TLR4/rs10759932*, *TLR4/rs11536889*, *TLR4/rs11536891* and *TLR4/rs11734502* (in complete linkage disequilibrium with *TLR4/rs4986790*) SNPs were included on all platforms. More detailed information is provided in the online supplement.

2.5 | Covariates

Information on important covariates such as sex, maternal and paternal allergies (yes/no), Dutch nationality (defined as both parents being born in the Netherlands, yes/no), parental education (low/medium/high, for details see online supplement), breastfeeding at age 12 weeks (yes/no), presence of older siblings (yes/no), daycare attendance at age 2 years, maternal smoking during pregnancy (yes/no), smoking in the child's home (yes/no), mould/damp spots in the living room and/or child's bedroom (yes/no), use of gas for cooking (yes/no) and furry pets in the child's home (cats, dogs, and/or rodents, yes/no) was obtained from the parent-completed questionnaires.

2.6 | Statistical analysis

Endotoxin levels were log-normally distributed, and therefore, mean values were expressed as geometric means with geometric standard deviations. Correlations between repeated endotoxin measurements (including also measurements performed during pregnancy and at age 1 year where available for the present study population) were expressed as Pearson correlation coefficients based on natural-log (ln) transformed data.

Overall associations of endotoxin levels measured in house dust samples collected at ages 3 months and 5-6 years with asthma and allergic sensitization prevalence at subsequent ages until age 17 were analysed by generalized estimation equations (GEE) models with a compound symmetry correlation matrix taking into account correlation between repeated observations within subjects.³⁷ We used a logit-link for both, asthma and allergic sensitization, despite the high prevalence of sensitization and the fact that the odds ratio than overestimates the relative risk because of convergence problems for models with a log-link. Age-specific association estimates were calculated from GEE models with exposure-age interaction terms. Analyses were performed with and without adjustment for the covariates listed above; associations between endotoxin levels at age 5-6 years and asthma were additionally adjusted for any allergic sensitization at age 4 to account for the nested case-control design.³⁴ Associations between endotoxin levels at age 5-6 years with allergic sensitization were not additionally adjusted for any allergic sensitization at age 4 as this may be on the causal pathway between endotoxin exposure and sensitization at later ages. Time-varying covariates were selected from the questionnaire that coincided best with the exposure period. We assessed modifications of associations with endotoxin levels in 3 months dust samples (numbers were too small for 5-6 year dust samples) by *CD14/rs2569190* and *TLR4* genotypes by adding interaction terms to the models and present genotype-specific association estimates from models with interaction terms. For SNPs with <10% of homozygous subjects for the minor allele, we combined all carriers of minor alleles. Since differential associations of house dust endotoxin have been suggested for atopic and non-atopic asthma,⁴ we also performed stratified analyses by allergic sensitization at or around the time of asthma assessment (using data on asthma collected at ages 1, 4, 8, 11 and 17 years and data on sensitization collected at ages 1, 4, 8, 12 and 16 years) with endotoxin levels in 3 months dust samples (again, numbers were too small for 5-6 year dust samples). Potential confounding by study arm (natural history study, intervention study—active, intervention study—placebo) and the presence of furry pets in the child's home, which are potential determinants of the exposure and therefore could be on the causal pathway between endotoxin exposure and the health outcomes studied, was assessed as part of a sensitivity analysis, where we additionally adjusted for these two variables. Ln-transformed endotoxin levels were used as continuous exposure variables in all analyses as smoothed curves (cubic splines estimated with SAS Proc GAM using the generalized cross validation function), suggested close to linear or at least monotonous relationships in most cases (Figures S2 and S3) and associations are presented as odds ratios (with 95% confidence intervals) for an interquartile range (IQR) increase in exposure on the ln-scale.

All analyses were performed with the Statistical Analysis System (SAS 9.2, Cary, NC, USA) for Windows. Statistical significance was defined by a two-sided α -level of 5%.

3 | RESULTS

3.1 | Population characteristics

Characteristics of the study population are presented in Table 1. The population is largely a non-farming population with only 1% reporting that they had lived on a farm during the child's first year of life and at age 5, respectively (data not shown). The percentage of children with allergic mothers is high in the total study population (Table 1) and in the subset with IgE data (Table S1). Table S2 provides the characteristics of the full PIAMA cohort and the subpopulations of the current study population with endotoxin data at ages 3 months and 5-6 years. The main difference between those who have endotoxin data and the full cohort is that participants of the intervention study (who have allergic mothers) are over-represented among those with endotoxin data. In addition, participants with endotoxin data at age 5-6 years, more often have highly educated parents. Other differences are generally small. The prevalence of asthma was between 10% and 12% at most ages; roughly, between one-third and two-thirds of the population were sensitized to any of the allergens tested at the different ages, and sensitization to food allergens was more common until the age of 4 years and sensitization to inhalant allergens thereafter (Figure 1).

3.2 | House dust endotoxin levels

Endotoxin loads for the child's mattresses (mostly new at the start of the study) increased during the course of the study. Endotoxin loads for living room floors were higher at age 5-6 years than at earlier ages, because of a higher percentage of samples collected on rugs at age 5-6 years, which yield larger amounts of dust. In contrast, average endotoxin concentrations per gram of mattress or living room floor dust were similar at ages 3 months and 5-6 years (Table 2). There was no correlation between endotoxin levels in repeated mattress dust samples and no correlation between endotoxin concentrations in repeated living room floor dust samples (Table S3), no correlation between endotoxin levels in mattress and living room floor dust samples for the same participant (Table S4), but some correlation between endotoxin loads in repeated living room floor dust samples ($r \leq 0.63$, Table S3).

3.3 | Associations of house dust endotoxin with asthma and allergic sensitization

Overall, after adjustment for covariates, endotoxin loads and concentrations in 3 months and 5-6 year dust samples were not associated with the prevalence of asthma until age 17 (Table 3). Age-specific association estimates suggest lower odds of asthma with increasing endotoxin levels in living room floor dust collected at age 3 months at ages 1-4 years (OR [95% CI] ranging from 0.70 [0.51-0.97] at age 1 to

TABLE 1 Participant characteristics

Covariate	n/N	(%)
Female sex	409/854	(47.9)
Allergic father	293/852	(34.4)
Allergic mother	750/854	(87.8)
Dutch nationality	716/793	(90.3)
Parental education		
Low	91/844	(10.8)
Medium	301/844	(35.7)
High	452/844	(53.6)
Breastfeeding \geq 12 wk	413/809	(51.1)
Older siblings	431/850	(50.7)
Daycare attendance at age 2 y	453/795	(57.0)
Mother smoking during pregnancy	120/842	(14.3)
Smoking in child's home ^a	179/845	(21.2)
Use of gas for cooking ^a	697/846	(82.4)
Mould/damp spots in living and/or bedroom ^a	74/832	(8.9)
Furry pets in the child's home ^a	499/848	(58.8)
Study arm		
Natural history	200/854	(23.4)
Intervention, active cover	345/854	(40.4)
Intervention, placebo cover	309/854	(36.2)
CD14/rs2569190 genotype		
CC	143/599	(23.9)
CT	327/599	(54.6)
TT	129/599	(21.5)
TLR4/rs2737190 genotype		
AA	282/599	(47.1)
GA	250/599	(41.7)
GG	67/599	(11.2)
TLR4/ rs10759932 genotype		
TT	461/599	(77.0)
TC	126/599	(21.0)
CC	12/599	(2.0)
TLR4/ rs11536889 genotype		
GG	458/599	(76.5)
GC	159/599	(21.5)
CC	12/599	(2.0)
TLR4/ rs11536891 genotype		
TT	421/599	(70.3)
TC	158/599	(26.4)
CC	20/599	(3.3)
TLR4/ rs11734502 genotype		
GG	539/599	(90.0)
GA	58/599	(9.7)
AA	2/599	(0.3)

^aDuring the 1st year of life.

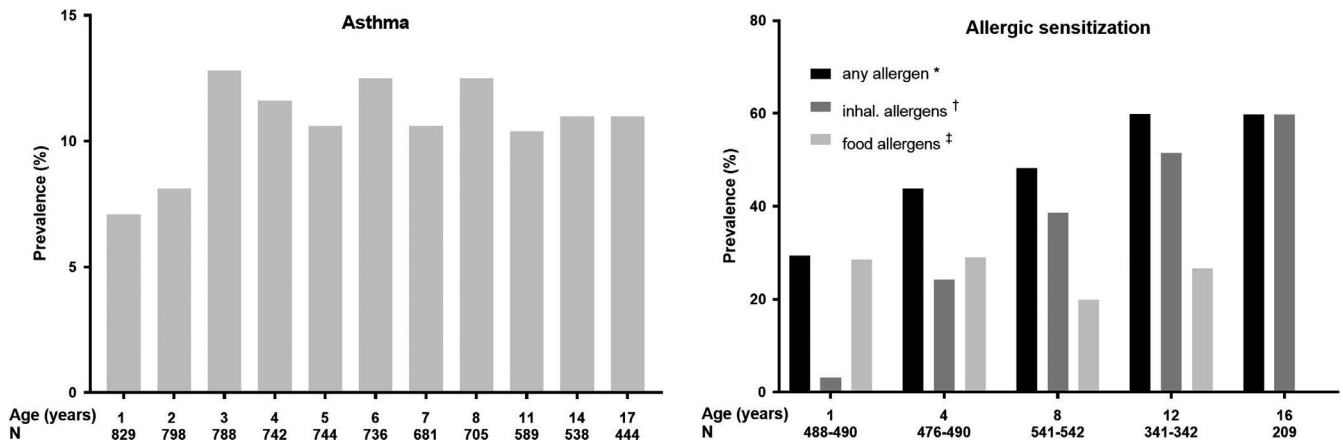


FIGURE 1 Prevalence of asthma and allergic sensitization from age 1 to 17 y

TABLE 2 Distribution of house dust endotoxin levels (in endotoxin units, EU) in mattress and living room floor dust

	Allergic mothers	Child's mattress				Living room floor ^a				
		GM (GSD)	P10	P90	N	GM (GSD)	P10	P90	N	Smooth floors
EU per m ²										
Pregnancy	100%	423 (4.8)	69	3435	190	508 (12.7)	22	17,656	129	79%
3 mo	100%	819 (3.2)	188	3547	610	495 (11.6)	18	13,248	612	80%
1 y	100%	1572 (3.4)	386	5174	91	328 (7.6)	20	5,080	124	85%
5-6 y	70%	2444 (2.5)	756	8765	344	2110 (10.0)	80	36,645	345	32%
EU per g dust										
Pregnancy	100%	11 733 (3.1)	2930	58,589	96	15 280 (4.4)	1690	143,255	87	69%
3 mo	100%	11 668 (2.7)	3627	41,308	494	14 093 (3.7)	2673	64,033	431	71%
1 y	100%	17 334 (2.7)	5350	62,625	81	12 580 (4.0)	2238	67,468	81	78%
5-6 y	70%	10 755 (2.1)	4539	28,416	345	14 569 (4.2)	3939	55,568	345	32%

^aWall-to-wall carpet and smooth floor otherwise until age 1; wall-to-wall carpet/rug > 4 m² and smooth floor otherwise at 5-6 y.

0.76 [0.57-1.00] at age 3), but not thereafter (Figure 2) and a higher risk of asthma at age 17 with higher living room floor dust endotoxin concentrations at age 5-6 years (OR [95% CI] 1.20 [0.99-1.45], Figures S4).

Also, there was no association between endotoxin levels at age 3 months and allergic sensitization until age 16 (Table 4 and Figure S5), but a positive association between endotoxin levels in living room floor dust collected at age 5-6 years and allergic sensitization to any allergen tested from age 8 onwards (Table 4). The positive associations with endotoxin levels in living room floor dust were largely driven by sensitization to inhalant allergens (Table S5), not sensitization to food allergens (Table S6), and fairly consistent across ages (Figures S6-S8).

3.4 | Effect modification

There were no statistically significant interactions between endotoxin exposure and *CD14/rs2569190* genotype for asthma and allergic sensitization and no consistent patterns of stronger (inverse) associations in carriers of specific genotypes (Table S7). There were

marginally significant ($P < .1$) interactions between endotoxin and the *TLR4/rs2737190*, *TLR4/rs11536889* and *TLR4/rs10759932* genotypes for asthma suggesting lower odds of asthma with higher mattress dust endotoxin levels among those with the GA *TLR4/rs2737190* genotype (Table S8) and GG *TLR4/rs11536889* and TC/CC *TLR4/rs10759932* genotypes (Table S9), respectively. There were no significant interactions of *TLR4* SNPs with allergic sensitization (Tables S8 and S10).

Overall associations of house dust endotoxin in 3 months dust samples with asthma until age 17 were not significantly different between subjects with and without allergic sensitization (Figure S9, all P -values of interaction terms were $>.23$). Interactions with allergic sensitization and phenotype were not assessed for age-specific associations as numbers became too small.

3.5 | Sensitivity analyses

Estimates of the overall associations of endotoxin exposure with asthma and allergic sensitization did not change after additional

TABLE 3 Crude and adjusted overall associations of endotoxin levels in mattress and living room floor dust samples at ages 3 mo and 5-6 y with asthma from age 1 to 17

	Crude			Crude ^a			Adjusted ^b		
	N (n) ^c	OR ^d	(95% CI)	N (n) ^c	OR ^d	(95% CI)	N (n) ^c	OR ^d	(95% CI)
Dust collected at age 3 mo and asthma at age 1-17									
Child's mattress									
EU per m ²	610 (5187)	0.98	(0.81-1.18)	503 (4554)	1.00	(0.81-1.23)	503 (4554)	0.96	(0.77-1.19)
EU per g dust	494 (4202)	0.88	(0.70-1.10)	412 (3729)	0.92	(0.71-1.19)	412 (3729)	0.88	(0.67-1.16)
Living room floor									
EU per m ²	612 (5175)	0.76	(0.58-1.00)	500 (4519)	0.80	(0.58-1.10)	500 (4519)	0.84	(0.60-1.18)
EU per g dust	431 (3664)	0.83	(0.63-1.10)	351 (3180)	0.83	(0.60-1.15)	351 (3180)	0.85	(0.61-1.18)
Dust collected at age 5-6 y and asthma at age 6-17									
Child's mattress									
EU per m ²	342 (1804)	1.09	(0.84-1.43)	290 (1937)	1.07	(0.80-1.43)	290 (1937)	1.14	(0.85-1.52)
EU per g dust	343 (1807)	1.11	(0.78-1.58)	290 (1937)	1.05	(0.73-1.52)	290 (1937)	1.00	(0.69-1.44)
Living room floor									
EU per m ²	343 (1807)	0.96	(0.67-1.38)	289 (1533)	0.94	(0.63-1.39)	289 (1533)	0.88	(0.55-1.39)
EU per g dust	343 (1807)	1.02	(0.85-1.23)	289 (1533)	1.02	(0.84-1.24)	289 (1533)	1.04	(0.81-1.34)

^aRestricted to participants with complete covariate data.

^bAdjusted for sex, paternal allergy, Dutch nationality, parental education, breastfeeding, older siblings, maternal smoking during pregnancy, parental smoking at home, use of gas for cooking, mould/dampness in the child's home. Associations with 5-6 year dust are additionally adjusted for allergic sensitization to any allergen at age 4 to account for the nested case-control design.

^cN = number of subjects; n = number of observations.

^dAssociations are presented for an interquartile range increase in exposure, which corresponds to 4.0 (child's mattress, load), 3.7 (child's mattress, concentration), 41.7 (living room floor, load), and 4.8-fold (living room floor, concentration) increases in exposure at age 3 mo and 2.9 (child's mattress, load), 2.6 (child's mattress, concentration), 32.8 (living room floor, load), and 3.3-fold (living room floor, concentration) increases in exposure at age 5-6 y.

adjustment for study arm and the presence of pets in the child's home (change in odds ratio was $\leq 6.1\%$).

4 | DISCUSSION

Overall, the results of our longitudinal analysis provide no evidence for an association of endotoxin levels in mattress dust with asthma and allergic sensitization until age 17 years. However, higher endotoxin concentrations in living room floor dust at age 3 months tended to be associated with a lower odds of asthma until age 4, but not thereafter, whereas house dust endotoxin levels measured at age 5-6 years were associated with a higher odds of sensitization to inhalant allergens at ages 8 and older.

This longitudinal analysis extends earlier findings of an inverse association between endotoxin load of living room floor dust samples collected early in life and asthma at age 4 and a lack of association between endotoxin load of mattress dust samples collected at the age of 5-6 years and asthma at age 6 from a previous cross-sectional analysis within the same cohort.^{33,38} The inverse associations of early life living room floor dust endotoxin exposure with asthma until age 4 (statistically significant for concentrations, marginally statistically significant ($P < .1$) for loads) are consistent with findings of previous European studies of preschool children aged 2 years³⁹

and 5 years,¹⁰ albeit inconsistent with regard to the relevant exposure site (ie living room floor vs maternal or child's mattress). Studies assessing associations of house dust endotoxin with asthma in children beyond the preschool age are cross-sectional (also within cohort studies) and except one study⁴⁰ lack information on early life exposure to endotoxin.^{4,6,16,17,20,38} Inverse associations between asthma and concurrent exposure to house dust endotoxin have been suggested by several studies in primary schoolchildren from the Netherlands³⁸ and North America,^{17,40} but not from New Zealand.¹⁶ A case-control study from Canada reported inverse associations of endotoxin with asthma in children aged 6-12 years, but not in adolescents aged 13-18 years.¹⁹ Apart from that study, studies in adolescents are scarce and findings of these studies, which are all not limited to adolescents, are mixed.^{4,6,20} A multicentre study of children aged 9-12 years from five European countries reported inverse associations between asthma and endotoxin load of living room floor dust.²⁰ In another European multicentre study of children aged 6-13 years from farming and non-farming households in rural areas, inverse associations of mattress dust endotoxin load were limited to atopic asthma⁴ and no significant association was found between asthma and endotoxin in living room floor dust in another study of children aged 5-14 years.⁶ Inverse associations have been found to be limited to atopic asthma in one study,⁴ but not in the present study and another study.⁴¹ Since differences in associations of house

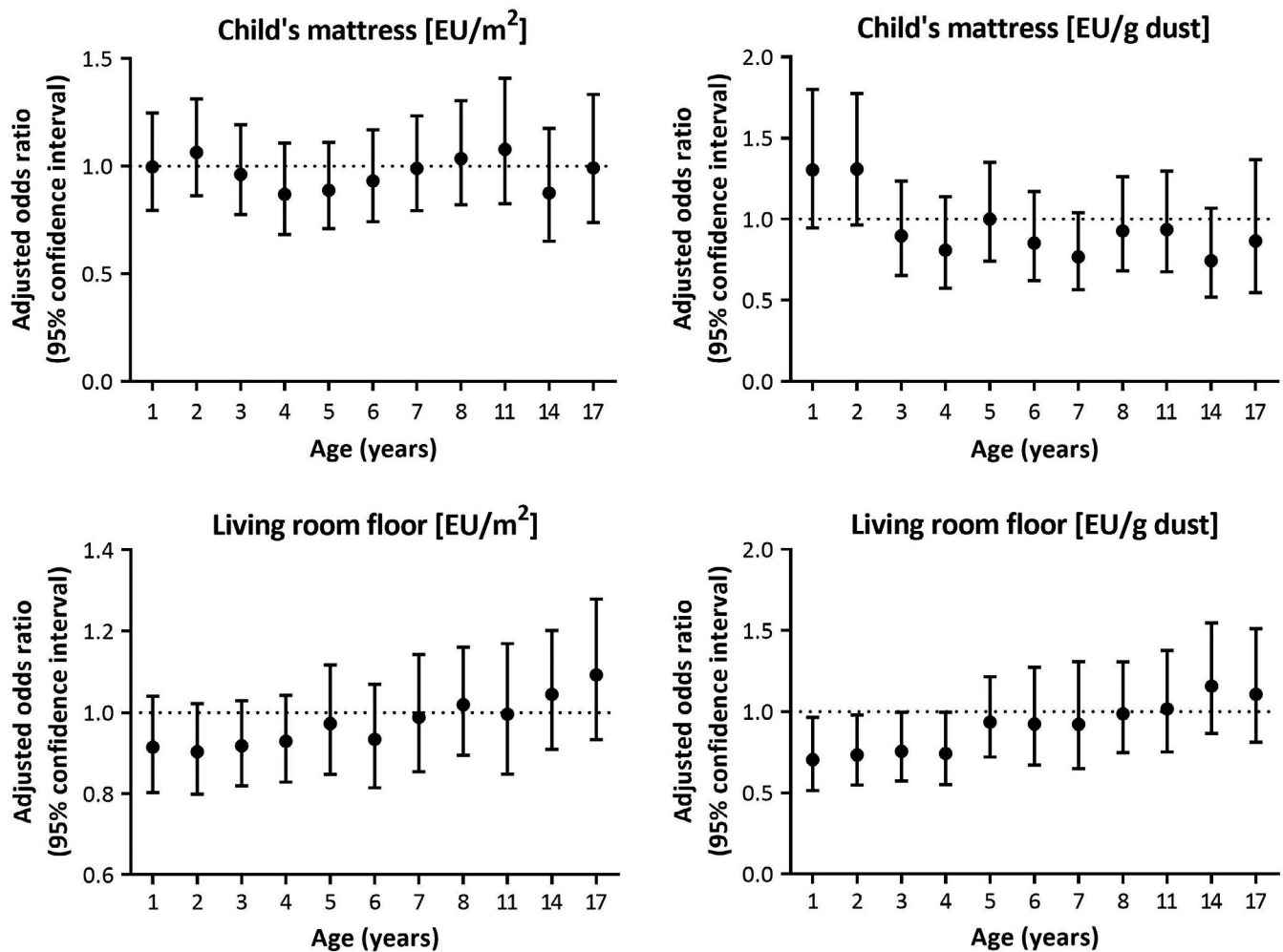


FIGURE 2 Age-specific adjusted[†] associations[‡] between asthma at ages 1 to 17 y and endotoxins in mattress and living room floor dust samples collected at age 3 mo. [†]Adjusted for sex, paternal allergy, Dutch nationality, parental education, breastfeeding, older siblings, maternal smoking during pregnancy, parental smoking at home, use of gas for cooking, mould/dampness in the child's home. [‡]Associations are presented for an interquartile range increase in exposure.

dust endotoxin with asthma between atopic and non-atopic subjects are often not taken into account, this may explain some of the heterogeneity in study findings. Taking all the evidence together, there is growing evidence for an inverse association between house dust endotoxin exposure early in life and asthma in preschool children, but not for such an association in schoolchildren and adolescents.

Studies assessing *CD14* and *TLR4* genotype-endotoxin interactions for asthma are scarce. Our findings on the interaction between endotoxin and *CD14/rs2569190* genotype are of interest, but inconclusive. They warrant further study as numbers are small in our study and the only other (case-control) study from Barbados, West Indies that found an antagonistic interaction between endotoxin levels in the home and *CD14/rs2569190* genotype with the TT genotype being protective for asthma among subjects with low exposure to endotoxin and associated with a higher risk of asthma among subjects with high exposure to endotoxin.⁴² Moreover, we found some evidence for a modification of associations between asthma and mattress dust endotoxin levels early in life by the *TLR4/rs2737190*, *TLR4/rs11536889* and *TLR4/rs10759932* genotypes that

are of interest. These SNPs that have been assessed as effect modifiers in farmer children previously, but no significant interactions have been observed in the earlier study.²⁸

Fewer studies assessed the relationship between house dust endotoxin exposure and allergic sensitization, but findings of these studies are more consistent and suggest inverse associations in infants aged 1-2 years,^{9,10} preschool¹⁰ and schoolchildren^{4,6} from Europe and the United States. Findings from a cohort from the United States were inconsistent with a lower odds of sensitization at age 7 in the second and third quartiles, but not in the fourth quartile of family room dust endotoxin concentrations measured early in life.⁵ Besides in the present study, positive associations with allergic sensitization have only been reported from a European multicentre at the age of 1 year.³⁹ The mechanisms underlying these positive associations are not clear. Moreover, differences in study design, definitions of outcome or exposure, and statistical analysis are no likely explanations for the opposite associations as there are no systematic differences between studies showing positive and inverse associations. Exposure levels are difficult to compare between

TABLE 4 Crude and adjusted overall associations of endotoxin levels in mattress and living room floor dust samples at ages 3 mo and 5-6 y and allergic sensitization^a from age 1 to 16

	Crude			Crude ^b			Adjusted ^c		
	N (n) ^d	OR ^e	(95% CI)	N (n) ^d	OR ^e	(95% CI)	N (n) ^d	OR ^e	(95% CI)
Dust collected at age 3 mo and sensitization at age 1-16									
Child's mattress									
EU per m ²	540 (1409)	0.89	(0.75-1.06)	457 (1226)	0.93	(0.77-1.12)	457 (1226)	0.98	(0.80-1.19)
EU per g dust	434 (1131)	0.88	(0.71-1.10)	371 (996)	0.91	(0.72-1.14)	371 (996)	0.97	(0.75-1.25)
Living room floor									
EU per m ²	546 (1424)	0.84	(0.68-1.04)	459 (1232)	0.87	(0.69-1.10)	459 (1232)	0.91	(0.71-1.16)
EU per g dust	387 (994)	1.00	(0.81-1.23)	326 (860)	1.03	(0.82-1.28)	326 (860)	1.05	(0.82-1.34)
Dust collected at age 5-6 y and sensitization at ages 8-16									
Child's mattress									
EU per m ²	306 (599)	1.05	(0.83-1.33)	271 (534)	1.14	(0.89-1.47)	271 (534)	1.13	(0.87-1.48)
EU per g dust	306 (599)	1.16	(0.88-1.53)	271 (534)	1.15	(0.86-1.53)	271 (534)	1.13	(0.84-1.52)
Living room floor									
EU per m ²	306 (599)	1.51	(1.10-2.09)	270 (533)	1.55	(1.09-2.19)	270 (533)	1.70	(1.17-2.47)
EU per g dust	306 (599)	1.15	(0.96-1.38)	270 (533)	1.19	(0.97-1.46)	270 (533)	1.24	(1.00-1.55)

$p < 0.05$ for all values that are presented in bold.

^aAgainst any of the allergens tested: house dust mite (*Dermatophagoides pteronyssinus*), cat, dog, birch, *Dactylis glomerata*, *Alternaria alternata*, milk and/or egg as available at the different ages.

^bRestricted to participants with complete covariate data.

^cAdjusted for sex, paternal allergy, Dutch nationality, parental education, breastfeeding, older siblings, maternal smoking during pregnancy, parental smoking at home, use of gas for cooking, mould/dampness in the child's home. Associations with allergic sensitization were not additionally adjusted for allergic sensitization to any allergen at age 4 despite the nested case-control design as this may be on the causal pathway between exposure and allergic sensitization after age 4.

^dN = number of subjects; n = number of observations.

^eAssociations are presented for an interquartile range increase in exposure, which corresponds to 4.0 (child's mattress, load), 3.7 (child's mattress, concentration), 41.7 (living room floor, load) and 4.8-fold (living room floor, concentration) increases in exposure at age 3 mo and 2.9 (child's mattress, load), 2.6 (child's mattress, concentration), 32.8 (living room floor, load) and 3.3-fold (living room floor, concentration) increases in exposure at age 5-6 y.

studies because of differences in sampling location, sample collection, extractions and analysis, but are also not a likely explanation of the inconsistent effects as inverse associations have not only been shown in farming populations as described above, where exposure levels are much higher than in non-farming population. Differences with regard to the form of endotoxin may explain the inconsistency in associations with endotoxin between the present study and earlier studies. Gram-negative bacteria have several forms of endotoxin that have different effects on asthma and allergic sensitization. For example, pentaacylated endotoxin has been found to be associated with lower risks, while hexaacylated endotoxin has been found to be associated with higher risks.^{43,44} However, neither in our study nor from earlier studies, information is available on different forms of endotoxin. Alternatively, co-exposure to other environmental organisms may explain inconsistencies between studies. In the GABRIELA study, it has been shown that the diversity of exposure to micro-organisms was associated with asthma; this was, however, not the case for sensitization.⁴⁵ Also, genetics may at least partly explain the contradicting findings. We could not confirm the findings of other studies suggesting an effect modification by single-nucleotide polymorphisms in the CD14 receptor,^{26,27} through which

endotoxin interacts with monocytes and macrophage, epithelial and endothelial cells.

Strengths of the present study include the longitudinal design with repeated assessments of health outcomes and exposures, which allowed us to assess exposure-response relationships at different ages from birth until adolescence, with exposure measurements preceding assessments of health outcomes, which has been an important limitation of previous cross-sectional studies. With the repeated exposure measurements were able to contribute to the currently limited evidence on the variability of house dust endotoxin levels over time, to show that living room floor dust endotoxin loads tend to be slightly more stable than mattress dust endotoxin levels in this age group, and to assess the relevance of exposure at different time-points. Nevertheless, we acknowledge that exposure measurements were largely limited to two time-points (3 months and 5-6 years) as the collection and analysis of dust samples is costly. Studies with more frequent endotoxin measurements are needed to assess the relevance of continuity or changes in exposure. It is currently not clear, which site is more relevant with regard to health outcomes, mattresses or living room floors, as associations have been shown for both exposure sites as discussed above. With

measurements of endotoxin levels in both mattress and living room floor dust, we demonstrated heterogeneity of endotoxin levels within homes, which means that one sample is not sufficient to characterize an individual's residential exposure. Limitations include the lack of exposure data beyond the age of 5-6 years. Several studies suggested that the effects of endotoxin on asthma and allergies are not limited to exposures during childhood, by showing associations with exposures in adulthood.^{8,11,46} There is some evidence for seasonal variation of endotoxin concentrations.⁴⁷ Season of dust sampling varied between study participants, but was not associated with the health outcomes studied. Therefore, this may have introduced some random exposure measurement error and may have biased associations towards the null. Moreover, the number of subjects with exposure at age 5-6 years is small. Furthermore, endotoxin in house dust may at least partly act as a proxy for other microbial exposures such as $\beta(1, 3)$ glucans⁴⁸ and other bacterial components⁴⁹ with immune-stimulatory properties. For the definition of asthma, we relied on questionnaire-reports rather than objective measures as objective measurements are not feasible in large epidemiological studies. Consequently, we cannot rule out reporting bias. Since study participants were unaware of their endotoxin exposure, outcome misclassification is most likely non-differential and (if anything) may have biased association estimates towards the null. Children of allergic mothers are over-represented in the current study sample, which is efficient as these are more prone to allergies, but may limit the generalizability of our findings to the entire cohort and the general population. Moreover, the number of children with non-allergic mothers is too small to allow separate analyses, but so far there is no evidence for potential effect modifications by parental allergy. Also, children of non-Dutch non-Western parents are under-represented and may limit the generalizability.

In conclusion, house dust endotoxin may have beneficial effects on asthma in preschool children of allergic mothers, which do not persist into adolescence. We did not find any evidence for a beneficial effect on allergic sensitization.

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CONFLICT OF INTEREST

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DATA AVAILABILITY STATEMENT

The PIAMA data are not freely accessible in the public domain, because this would be in conflict with the agreement between the PIAMA study team and the PIAMA participants. However, the data underlying the findings presented in this paper are available on request. Requests can be submitted to the PIAMA Principal Investigators (see at <http://piama.iras.uu.nl/index-en.php#collaboration> more for information).

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REFERENCES

1. World Health Organization. Asthma. 2017 August 31, 2017. <https://www.who.int/news-room/fact-sheets/detail/asthma>. Accessed January 6, 2020
2. Burbank AJ, Sood AK, Kesic MJ, Peden DB, Hernandez ML. Environmental determinants of allergy and asthma in early life. *J Allergy Clin Immunol*. 2017;140(1):1-12.
3. Los H, Postmus PE, Boomsma DI. Asthma genetics and intermediate phenotypes: a review from twin studies. *Twin Res*. 2001;4(2):81-93.
4. Braun-Fahrländer C, Riedler J, Herz U, et al. Environmental exposure to endotoxin and its relation to asthma in school-age children. *N Engl J Med*. 2002;347(12):869-877.
5. Celedón JC, Milton DK, Ramsey CD, et al. Exposure to dust mite allergen and endotoxin in early life and asthma and atopy in childhood. *J Allergy Clin Immunol*. 2007;120(1):144-149.
6. Gehring U, Bischof W, Fahlbusch B, Wichmann HE, Heinrich J. House dust endotoxin and allergic sensitization in children. *Am J Respir Crit Care Med*. 2002;166(7):939-944.
7. Thorne PS, Kulhánková K, Yin M, Cohn R, Arbes SJ, Zeldin DC. Endotoxin exposure is a risk factor for asthma: the national survey of endotoxin in U.S. Housing. *Am J Respir Crit Care Med*. 2005;172(11):1371-1377.
8. Gehring U, Bischof W, Schlenvoigt G, et al. Exposure to house dust endotoxin and allergic sensitization in adults. *Allergy*. 2004;59(9):946-952.
9. Gereda JE, Leung D, Thatayatikom A, et al. Relation between house-dust endotoxin exposure, type 1 T-cell development, and allergen sensitisation in infants at high risk of asthma. *Lancet*. 2000;355(9216):1680-1683.
10. Illi S, Weber J, Zutavern A, et al. Perinatal influences on the development of asthma and atopy in childhood. *Ann Allergy Asthma Immunol*. 2014;112(2):132-139.e1.
11. Portengen L, Preller L, Tielen M, Doekes G, Heederik D. Endotoxin exposure and atopic sensitization in adult pig farmers. *J Allergy Clin Immunol*. 2005;115(4):797-802.
12. Howard E, Orhurhu V, Huang L, Guthrie B, Phipatanakul W. The impact of ambient environmental exposures to microbial products on asthma outcomes from birth to childhood. *Curr Allergy Asthma Rep*. 2019;19(12):59.
13. Mendy A, Gasana J, Vieira ER, et al. Endotoxin exposure and childhood wheeze and asthma: a meta-analysis of observational studies. *J Asthma*. 2011;48(7):685-693.
14. Gehring U, Bolte G, Borte M, et al. Exposure to endotoxin decreases the risk of atopic eczema in infancy: a cohort study. *J Allergy Clin Immunol*. 2001;108(5 Part 1):847-854.

15. Gillespie J, Wickens K, Siebers R, et al. Endotoxin exposure, wheezing, and rash in infancy in a New Zealand birth cohort. *J Allergy Clin Immunol*. 2006;118(6):1265-1270.
16. Wickens K, Lane JM, Fitzharris P, et al. Farm residence and exposures and the risk of allergic diseases in New Zealand children. *Allergy*. 2002;57(12):1171-1179.
17. Carlsten C, Ferguson A, Dimich-Ward H, et al. Association between endotoxin and mite allergen exposure with asthma and specific sensitization at age 7 in high-risk children. *Pediatr Allergy Immunol*. 2011;22(3):320-326.
18. El Sharif N, Douwes J, Hoet P, Nemery B. Childhood asthma and indoor aeroallergens and endotoxin in Palestine: a case-control study. *J Asthma*. 2006;43(3):241-247.
19. Lawson JA, Dosman JA, Rennie DC, et al. Endotoxin as a determinant of asthma and wheeze among rural dwelling children and adolescents: a case-control study. *BMC Pulm Med*. 2012;12:56.
20. Gehring U, Strikwold M, Schram-Bijkerk D, et al. Asthma and allergic symptoms in relation to house dust endotoxin: Phase Two of the International Study on Asthma and Allergies in Childhood (ISAAC II). *Clin Exp Allergy*. 2008;38:1911-1920.
21. Tulic MK, Wale JL, Holt PG, Sly PD. Modification of the inflammatory response to allergen challenge after exposure to bacterial lipopolysaccharide. *Am J Respir Cell Mol Biol*. 2000;22(5):604-612.
22. Lapa e Silva JR, Possebon da Silva MD, Lefort J, Vargaftig BB. Endotoxins, asthma, and allergic immune responses. *Toxicology*. 2000;152(1-3):31-35.
23. Ulmer AJ. Biochemistry and cell biology of endotoxins. *Int J Occup Environ Health*. 1997;3:8-17.
24. Dubin W, Martin TR, Swoveland P, et al. Asthma and endotoxin: lipopolysaccharide-binding protein and soluble CD14 in bronchoalveolar compartment. *Am J Physiol*. 1996;270(5 Pt 1):L736-L744.
25. Arbour NC, Lorenz E, Schutte BC, et al. TLR4 mutations are associated with endotoxin hyporesponsiveness in humans. *Nat Genet*. 2000;25(2):187-191.
26. Eder W, Klimecki W, Yu L, et al. Opposite effects of CD 14/-260 on serum IgE levels in children raised in different environments. *J Allergy Clin Immunol*. 2005;116(3):601-607.
27. Simpson A, John SL, Jury F, et al. Endotoxin exposure, CD14, and allergic disease: an interaction between genes and the environment. *Am J Respir Crit Care Med*. 2006;174(4):386-392.
28. Eder W, Klimecki W, Yu L, et al. Toll-like receptor 2 as a major gene for asthma in children of European farmers. *J Allergy Clin Immunol*. 2004;113(3):482-488.
29. Brunekreef B, Smit J, de Jongste J, et al. The prevention and incidence of asthma and mite allergy (PIAMA) birth cohort study: design and first results. *Pediatr Allergy Immunol*. 2002;13(Suppl 15):55-60.
30. Wijga AH, Kerkhof M, Gehring U, et al. Cohort profile: the prevention and incidence of asthma and mite allergy (PIAMA) birth cohort. *Int J Epidemiol*. 2014;43(2):527-535.
31. Gehring U, de Jongste JC, Kerkhof M, et al. The 8-year follow-up of the PIAMA intervention study assessing the effect of mite-impermeable mattress covers. *Allergy*. 2012;67(2):248-256.
32. Pinart M, Benet M, Annesi-Maesano I, et al. Comorbidity of eczema, rhinitis, and asthma in IgE-sensitized and non-IgE-sensitized children in MeDALL: a population-based cohort study. *Lancet Respir Med*. 2014;2(2):131-140.
33. Douwes J, van Strien R, Doekes G, et al. Does early indoor microbial exposure reduce the risk of asthma? The Prevention and Incidence of Asthma and Mite Allergy birth cohort study. *J Allergy Clin Immunol*. 2006;117(5):1067-1073.
34. Gehring U, Heinrich J, Hoek G, et al. Bacteria and mould components in house dust and children's allergic sensitisation. *Eur Respir J*. 2007;29(6):1144-1153.
35. Schram D, Doekes G, Boeve M, et al. Bacterial and fungal components in house dust of farm children, Rudolf Steiner school children and reference children - the PARSIFAL Study. *Allergy*. 2005;60(5):611-618.
36. Douwes J, Versloot P, Hollander A, Heederik D, Doekes G. Influence of various dust sampling and extraction methods on the measurement of airborne endotoxin. *Appl Environ Microbiol*. 1995;61(5):1763-1769.
37. Diggle PJ, Liang KY, Zeger SL. *Analysis of longitudinal data*. Oxford: Clarendon Press; 1994.
38. Tischer C, Gehring U, Chen CM, et al. Respiratory health in children, and indoor exposure to (1,3)-beta-D-glucan, EPS mould components and endotoxin. *Eur Respir J*. 2011;37(5):1050-1059.
39. Karvonen AM, Hyvärinen A, Gehring U, et al. Exposure to microbial agents in house dust and wheezing, atopic dermatitis and atopic sensitization in early childhood: a birth cohort study in rural areas. *Clin Exp Allergy*. 2012;42(8):1246-1256.
40. Sordillo JE, Hoffman EB, Celedon JC, Litonjua AA, Milton DK, Gold DR. Multiple microbial exposures in the home may protect against asthma or allergy in childhood. *Clin Exp Allergy*. 2010;40(6):902-910.
41. Thorne PS, Mendy A, Metwali N, et al. Endotoxin exposure: predictors and prevalence of associated asthma outcomes in the United States. *Am J Respir Crit Care Med*. 2015;192(11):1287-1297.
42. Zambelli-Weiner A, Ehrlich E, Stockton ML, et al. Evaluation of the CD14/-260 polymorphism and house dust endotoxin exposure in the Barbados Asthma Genetics Study. *J Allergy Clin Immunol*. 2005;115(6):1203-1209.
43. Brix S, Eriksen C, Larsen JM, Bisgaard H. Metagenomic heterogeneity explains dual immune effects of endotoxins. *J Allergy Clin Immunol*. 2015;135(1):277-280.
44. Hadina S, Weiss JP, McCray PB Jr, Kulhankova K, Thorne PS. MD-2-dependent pulmonary immune responses to inhaled lipooligosaccharides: effect of acylation state. *Am J Respir Cell Mol Biol*. 2008;38(6):647-654.
45. Ege MJ, Mayer M, Normand A-C, et al. Exposure to Environmental Microorganisms and Childhood Asthma. *N Engl J Med*. 2011;364(8):701-709.
46. Carnes MU, Hoppin JA, Metwali N, et al. Dust endotoxin levels are associated with adult asthma in a U.S. farming population. *Ann Am Thorac Soc*. 2017;14(3):324-331.
47. Giovannangelo M, Gehring U, Nordling E, et al. Determinants of house dust endotoxin in three European countries - the AIRALLERG study. *Indoor Air*. 2007;17(1):70-79.
48. Rylander R, Lin R. (1->3)-beta-D-glucan - relationship to indoor air-related symptoms, allergy and asthma. *Toxicology*. 2000;152(1-3):47-52.
49. van Strien RT, Engel R, Holst O, et al. Microbial exposure of rural school children, as assessed by levels of N-acetyl-muramic acid in mattress dust, and its association with respiratory health. *J Allergy Clin Immunol*. 2004;113(5):860-867.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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