

Childhood asthma and early life exposure to indoor allergens, endotoxin and $\beta(1,3)$ -glucans

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Summary

Background Divergent results have been reported regarding early life exposure to indoor environmental agents and the risk of asthma and allergic sensitization later in life.

Objective To assess whether early exposure to indoor allergens, $\beta(1,3)$ -glucans and endotoxin modifies the risk of allergic diseases at 10 years of age.

Methods The concentrations of mite, cat and dog allergens, endotoxin and $\beta(1,3)$ -glucans were determined in dust from the homes of 260 two-year-old children with lung function measured at birth (tidal flow volume loops) in the Environment and Childhood Asthma study in Oslo. At 10 years, the health status was assessed in a follow-up study including a structured interview of the parents and an extended clinical examination.

Results Cat and dog keeping at 2 years of age was reported in 6.5% and 5.5% of the families, respectively. Mite allergens were detected in only 4/260 dust samples. The adjusted odds ratio for asthma at age 10 was 1.20 (95% confidence interval: 1.01–1.43) and 1.22 (1.02–1.46) for bronchial hyperresponsiveness (BHR) per 10 $\mu\text{g/g}$ dust increase in cat allergen exposure at 2 years of age. No association was seen with allergic sensitization. Moreover, endotoxin and $\beta(1,3)$ -glucan exposure did not modify the risk of asthma or allergic sensitization. None of the measured environmental factors were associated with lung function at 10 years of age or a relative change in lung function from birth.

Conclusion In a community with a low prevalence of pet keeping and low mite allergen levels, exposure to cat allergens early in life increased the risk of late childhood asthma and BHR, but not the risk of allergic sensitization. No risk modification was seen for dog allergens, endotoxin and $\beta(1,3)$ -glucans.

Keywords $\beta(1,3)$ -glucan, childhood asthma, cohort study, endotoxin, indoor allergens

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Introduction

Early life exposure to indoor allergens and microbial products may influence the development of allergic disease [1–3]. However, the majority of studies reporting associations between exposure to indoor environmental agents and allergic diseases have been cross-sectional [4–8]. Moreover, associations between indoor allergen exposure and asthma have usually only been inferred indirectly via allergic sensitization as a risk factor for asthma [6, 7, 9, 10]. So far, no published longitudinal population-based studies have demonstrated a direct link

between early life allergen exposure and asthma later in childhood [11–13]. In high-risk children, however, such associations have been reported for mite allergens and asthma [13–15].

Contrary to allergen exposure, in a meta-analysis, exposure to pets was found to increase the risk of asthma and wheezing in children over 6 years of age [16]. However, keeping a pet does not always imply very high allergen exposure levels, as the exposure to pets is influenced by geographic differences and cultural habits. Furthermore, pet allergen exposure at home is also

influenced by the prevalence of pet keeping in the society, because of indirect allergen exposure in public places [17–19]. Also, the associations between pet allergen exposure and allergic diseases may be overshadowed by the effects of mite allergen exposure in areas where the climate is favourable for mite growth [2]. Finally, possible protective effects from exposure to microbial agents have been suggested, such as modulating effects on the immune system by endotoxin early in life causing tolerance or non-allergic immune responses to allergens [14, 20]. Early life exposure to endotoxin and fungal agents has been found to be inversely associated with the risk of atopic asthma [21] but has also been reported to increase the risk of wheezing [14, 22].

Reduced lung function shortly after birth [23] or in early infancy [24] predicts airflow limitation in later childhood, which in turn is a strong pre-disposing factor for asthma [23, 24]. It has been suggested that there is a developmental window of susceptibility in early childhood during which environmental exposures can disrupt normal growth trajectories [24, 25]. It is therefore likely that early life exposures may influence lung function and asthma later in childhood, as was demonstrated in the German MAS study, where sensitization and exposure to high levels of mite and cat allergens in the first 3 years of life had a much stronger impact on the loss of lung function at school age than exposure later in childhood [9].

In order to assess modification of the natural history of disease, it is essential to consider pre-disposition for allergic disease development. In the Environment and Childhood Asthma (ECA) birth cohort study in Oslo, we found several risk factors at birth that may predict asthma 10 years later. These included reduced lung function [23], markers of family stress and socio-economic factors, parental allergic disease as well as alcohol consumption during pregnancy, which could be accounted for in risk modification assessment. We therefore aimed to assess whether exposure to indoor allergens, $\beta(1,3)$ -glucans and endotoxin in the homes of the children at 2 years of age modified the risk of asthma, allergic sensitization, BHR and lung function at 10 years of age.

Materials and methods

Study design and subjects

The present study is part of the prospective birth cohort 'The ECA' study established in Oslo in 1992–1993 [26]. The present study concerns those healthy infants included at birth who had lung function measured at a mean age of 2.7 (SD $\pm$ 0.9) days by tidal flow volume (TFV) loops [27]. Parents completed detailed questionnaires regarding the family history of allergic diseases and environmental exposures at birth and thereafter every 6 months for the

first 2 years of life [26]. At 2 years of age, all enrolled and participating families were invited to submit dust samples from their home.

At 10 years of age, 616/802 children (77%) attended a follow-up study with two clinical examinations performed 2–7 days apart and at least 4 weeks after any suspicion of a respiratory tract infection. Examinations included measures of lung function and bronchial hyper-responsiveness (BHR), allergological investigations and a structured parental interview [23, 26], with detailed questions regarding environmental exposure, life style and diseases (index child and family). The 616 children with lung function measured at birth who attended the 10-year examination were representative of the entire birth cohort ($n = 3754$) as there were no significant differences at birth with respect to gender, parental age, parental asthma, eczema or rhinoconjunctivitis, maternal smoking during pregnancy, pet keeping, parental education, paternal employment rate, family income or number of siblings [26].

All the study doctors and technicians involved in the 10-year assessments were blinded to the results of the lung function measurements at birth and to other information obtained from the participants.

Written informed consent was obtained from the parents, and the study was approved by the Regional Committee for Ethics in Medical Research and the Norwegian Data Inspectorate and registered in the Norwegian Biobank Registry.

The invitation to submit house dust samples at 2 years prompted a response from 260 of the 616 children who attended the follow-up study at 10 years of age. Except for higher parental education level, the 260 children with dust samples were similar to the 365 non-responders with regard to birth characteristics and pet keeping during the first 2 years of life (Table 1). Regarding characteristics at 10 years of age, the non-included children were more often sensitized to cat (Table 1) but were otherwise comparable.

Dust sampling and extractions at 2 years

The dust sample was collected by the parents according to detailed written instructions. In brief, the floor of the living area of the house was vacuumed using a new vacuum cleaner bag, and the collected dust was sent to the Norwegian Institute of Public Health and stored at -20°C until extraction and analysis. Endotoxin, allergens and $\beta(1,3)$ -glucans were extracted sequentially from unsieved dust. Endotoxin was extracted in pyrogen-free water with 0.05% Tween-20 and allergens in phosphate-buffered saline (PBS) with 0.05% Tween-20 (PBT) at room temperature. Glucans were extracted in PBT at 120°C . Details of dust sampling and extraction of allergens, endotoxins and $\beta(1,3)$ -glucans are given in the on-line supplement.

Table 1. Characteristics at birth and outcome variables at age 10 for included ($n = 260$) and non-included children ($n = 356$)

	Included	Non-included	P
Characteristics at birth			
Gender (% girls)	49.2	43.8	0.2
First-born child	53.1	47.2	0.2
Parental asthma (%)	13.1	11.2	0.5
Parental rhinoconjunctivitis (%)	33.1	27.2	0.1
Alcohol ever during first trimester (%)	52.3	50.8	0.7
Family network (mean of category)*	2.20	2.05	0.06
Babysitter daytime (mean of category) [†]	5.61	5.06	0.07
Parental education (% university) [‡]	68.0	50.0	<0.001
t_{PEF}/t_E (mean (95% CI))	0.31 (0.29–0.32)	0.33 (0.31–0.34)	0.06
Expiratory volume V_E [mean (95% CI)]	24.7 (24.1–25.2)	25.2 (24.7–25.7)	0.1
Pet keeping			
Dog at age 2 years (%)	5.5	5.3	0.9
Dog at 10 years (%)	10.5	17.0	0.01
Cat at age 2 years (%)	6.5	6.1	0.8
Cat at 10 years (%)	18.5	20.5	0.5
Reported pet avoidance (%) [§]	32.7	26.7	0.1
Outcome variables at 10 years of age			
FEV ₁ % predicted [mean (95% CI)]	99.8 (98.6–100.9)	100.8 (99.8–101.7)	0.2
FEF ₅₀ % predicted [mean (95% CI)]	99.5 (97.0–102)	102.0 (99.7–104.3)	0.2
PD20 ≤ 8 μ mol methacholine (%)	29.6	28.4	0.7
History of asthma (%)	18.5	21.5	0.4
Current asthma (%)	11.2	11.0	0.9
Allergic sensitization (%)	38.6	37.8	0.8
Sensitization to inhalant allergens (%)	34.6	35.2	0.9
Sensitization to food allergens (%)	15.9	12.4	0.2
Sensitized to cat (%)	10.4	16.9	0.02
Sensitized to dog (%)	13.9	14.5	0.9
Sensitized to mite (%)	8.0	11.5	0.2

*How easy will it be for the parents to contact family or friends for questions related to care of the baby?

[†]Babysitter daytime: how easy is it to get a babysitter during daytime? * and [†] were reported on a visual analogue scale from 1 to 10 (1 being very easy and 10 impossible).

[‡]Parental education in three categories: 1, 9–10 years compulsory (± 1 –2 years county college); 2, college; 3, university.

[§]Reported in parental interview at the 10-year follow-up, due to allergic disease already present in the family or to prevent the children from developing allergy or asthma.

Analyses of cat, dog and mite allergens, endotoxin and $\beta(1,3)$ -glucans (see supporting information for details)

The concentrations of dog allergen (*Canis familiaris* Can f1), cat allergen (*Felis domesticus* Fel d1) and mite allergen (*Dermatophagoides pteronyssinus* Der p 1) were determined by an enzyme-linked immunosorbent assay according to the manufacturer's instructions (Indoor Biotechnologies, Clewyd, UK) [28]. The lower detection limits were determined from the standard curves and were set to 12.5 ng/mL for Can f1, 5 ng/mL for Fel d1 and 6.25 ng/mL for Der p 1. For statistical purposes, concentrations below the detection limit were given a value of two-thirds of the lower detection limit for the assays (59 samples for dog allergen, 96 for cat allergen and 256 for mite allergen). Results were given as microgram allergen per gram dust.

Endotoxin was analysed using the kinetic chromogenic Limulus Amebocyte Lysate (LAL) test, using the same batch of LAL reagents and standards for all analyses [BioWhittaker, Walkersville, USA, LAL lysate lot no. FL119G, lipopolysaccharides (LPS) standard lot no. FL0835]. Thirty percent of the samples were tested in duplicate, with a mean coefficient of variation of 4.8%. Concentration was expressed as endotoxin units (EU) per milligram dust.

$\beta(1,3)$ -Glucans were measured using an inhibition enzyme immuno assay (EIA) [29] using laminarin (Bio-Chemika; Sigma-Aldrich, St Louis, MO, USA) in PBS and affinity-purified anti- $\beta(1,3)$ -glucan antibodies (Batch 9819, provided by Dr G. Doekes, IRAS, Utrecht, the Netherlands) in PBT with gelatine. Optical density was read at 490 nm, and the concentration was expressed as μ g $\beta(1,3)$ -glucan per gram dust.

Clinical examination and outcomes

Lung function at birth was measured by the SensorMedics 2600 (SensorMedics Corp, Anaheim, CA, USA) as described elsewhere [27]. Briefly, measurements were performed with the infants awake, through a pneumotachograph with a flow range of 0–10 L/min and dead space 2.4 mL (8311 series; Hans Rudolph, MO, USA). The TFV ratio time until peak tidal expiratory flow to total expiratory time (t_{PTEF}/t_E) was calculated.

At 10 years of age, lung function was measured by forced expiratory flow volume loops according to European standards [30] on a Sensor Medics Vmax 20c (SensorMedics Diagnostics, Yorba Linda, CA, USA) on two occasions within a week. The reported values were the best values obtained of these two efforts and included forced expiratory volume in 1 s (FEV_1) and forced expiratory flow at 50% of forced vital capacity (FEF_{50}) as percent predicted (reference values from healthy children in the 10-year follow-up of the ECA study obtained by methods described by Stanojevic et al. [31]).

BHR was measured using a methacholine challenge test performed according to international guidelines [32] using an inspiration-triggered Spira nebulizer (Spira Respiratory Care Centre, Hämeenlinna, Finland). BHR was defined by the cumulative dose of inhaled metacholine ($\leq 8 \mu\text{mol}$) causing a 20% decrease in FEV_1 from the baseline value (PD_{20}).

A standardized exercise test by treadmill running was performed with an exercise load equivalent to 95% of the maximal heart rate for at least 4 min [33]. FEV_1 was measured before, and 3, 6, 10 and 15 min after running. The exercise challenge test was considered positive with an $\geq 10\%$ reduction of baseline FEV_1 measured 3–20 min after running ceased.

A *history of asthma* was defined by the confirmation of at least two of the three following criteria: asthma symptoms, doctor's diagnosis of asthma and/or use of asthma medication before 10 years of age [26].

A diagnosis of *current asthma* at 10 years of age was established when a child with a history of asthma had either had symptoms or used asthma medication during the last 12 months and/or had a positive exercise challenge test at the 10-year follow-up visit [26]. Of all the children with current asthma in the 10-year follow-up, 74.6% had used asthma medication during the last 12 months and 42.6% had a positive exercise challenge test (61.5% with both criteria fulfilled).

Skin prick tests (SPT) were performed at age 10 [26] with Soluprick[®] allergens (ALK-Abello, Denmark), according to international standards [34]. Histamine (10 mg/mL) was used as a positive control. A test was considered positive with a mean weal diameter ≥ 3 mm larger than the negative control (0.9% NaCl) (< 1 mm in all subjects). Specific serum IgE (sIgE) was measured using

a radioallergosorbent fluorescence immunoassay (ImmunoCAP[®] system, Phadia, Uppsala, Sweden) and defined as positive when $sIgE \geq 0.35$ kU/L.

Allergic sensitization was defined by at least one positive SPT and/or sIgE to at least one of the following allergens: domestic mites (*Dermatophagoides pteronyssinus* and *Dermatophagoides farinae*), German cockroach, dog, cat and rabbit dander, birch, timothy (grass) and mugwort pollens, moulds (*Cladosporium herbarum* and *Alternaria alternata*), egg white, milk, peanut and codfish. *Sensitization to inhalant allergens* was defined by a positive SPT/sIgE to any of the inhalant allergens listed above (excluding the food allergens).

Co-variables (predictors at birth of asthma at age 10)

All analyses were adjusted for the birth variables previously identified as risk factors for asthma in this particular cohort (K. C. Lødrup Carlsen, P. Mowinckel, B. Granum, K. H. Carlsen, unpublished data): gender, how easy it was to get a babysitter during daytime, family network, any alcohol consumed during pregnancy, parental rhinoconjunctivitis, parental education, t_{PTEF}/t_E and V_E (expiratory volume). t_{PTEF}/t_E and V_E were dichotomized according to the median values of 0.29 and 24.2, respectively. Except for t_{PTEF}/t_E and V_E from the lung function measurements at birth, the other covariates were retrieved from questionnaires at birth.

Statistical analyses

Categorical variables were compared using Pearson's χ^2 -test, and continuous variables using one-way ANOVA. For calculation of change in lung function from birth (t_{PTEF}/t_E) to age 10 (FEV_1 or FEF_{50}), z-scores based on mean and SD of the healthy subjects were calculated. The z-scores describe each child's position relative to the healthy individuals in the population. The reference values for the z-scores at birth were calculated for all children (as they were healthy at the time) and at 10 years for all children without any lower respiratory tract infections during the first 2 years of life and without a history of asthma [35]. The predictor variables of interest were mite, cat and dog allergens, endotoxin and $\beta(1,3)$ -glucan exposure at 2 years of age. However, mite allergen concentrations were above the assay detection limit in only 4/260 samples, and were therefore excluded from further analyses. The concentrations of allergens, endotoxin and $\beta(1,3)$ -glucans in homes with and without pets were compared using the non-parametric Mann-Whitney U-test, and correlations were given by Spearman's ρ coefficient. The outcome variables FEV_1 and FEF_{50} (at age 10) and change in lung function from birth to age 10 were used as continuous variables in linear regression models.

The condition of normally distributed residuals of the dependent variable was fulfilled for each of the independent variables in the models.

Logistic regression models were used for the categorical outcome variables current asthma and a history of asthma, allergic sensitization and BHR ($PD_{20} \leq 8 \mu\text{mol}$ methacholine). The condition of linearity was tested using a fractional polynomial analysis [36] that supported treating the cat and dog allergens, endotoxin and $\beta(1,3)$ -glucans variables as continuous and linear in the model. The results of the logistic regression analyses were reported as the odds ratios (OR) per $10 \mu\text{g}$ increase in allergen exposure. The final multivariate model was built as described by Hosmer and Lemeshow [37]. Furthermore, all models were tested for confounding and interactions. The limit of statistical significance was set to 0.05 (5%). A binary logistic regression analysis with a sample size of 260 observations has a statistical power of about 70% for the observed OR under the assumption that the independent variables have an R^2 of at least 0.30. Statistical analyses were performed using Statistical Package for Social Sciences (SPSS version 14.0, SPSS Inc., Chicago, IL, USA) and Statistical Analysis System (SAS version 9.1.3, SAS Institute Cary, NC, USA) and Number Cruncher Statistical System (NCSS version 2007, Kaysville, UT, USA).

Results

At 10 years of age, 29/260 (11.2%) children had current asthma, 48 (18.5%) had history of asthma and 77 (29.6%) had BHR ($PD_{20} \leq 8 \mu\text{mol}$) (Table 1). The mean values of FEV_1 and FEF_{50} % predicted were 99.8 and 99.5, respectively (Table 1).

Among the 29 children (20 boys and nine girls) with current asthma, 16 (55%) had BHR ($PD_{20} \leq 8 \mu\text{mol}$) and 19 (66%) were sensitized to at least one allergen. Overall, 72.4% had used asthma medication during the last 12 months and 46.2% had a positive exercise challenge test (66.7% with both). The mean body mass index for boys with current asthma was 17.7 (2.7 SD) and 17.7 (3.3 SD) for girls, similar to 17.8 (2.5 SD) and 17.3 (2.1 SD) for boys and girls who had never had asthma, respectively ($P > 0.6$ for both boys and girls).

Cat, dog and mite allergen, endotoxin and $\beta(1,3)$ -glucan exposure levels

Cat allergen concentrations were significantly higher in homes with cats compared with homes without cats [median (25th–75th percentile): 31.6 (2.3–65.5) and 0.2 (0.1–0.3) μg Fel d1/g dust, respectively, $P < 0.001$, Fig. 1]. The corresponding dog allergen concentrations were 54.2 (27.0–94.1) and 0.1 (0.1–0.3) μg Can f1/g, respectively ($P < 0.001$). The concentrations of $\beta(1,3)$ -glucans and endotoxin did not differ between homes with pets [2553

(2023–3362) μg $\beta(1,3)$ -glucans/g dust and 22 (14–35) EU/mg dust] and without [2795 (2100–3576), $P = 0.5$ and 17 (9–42), $P = 0.1$, respectively] (Fig. 1). The correlations between dog, cat, endotoxin and $\beta(1,3)$ -glucan levels were low for all groups (all $\rho \leq 0.17$). Mite allergen levels (Der p 1) were above the detection limit only in four samples.

Exposure to environmental agents and allergic sensitization at age 10

Overall, 88.6% of the children with allergic sensitization to any of the allergens tested ($n = 98$) were sensitized to inhalant allergens ($n = 90$). Only eight children were sensitized to food allergens alone. None of the environmental agents measured at 2 years of age [cat or dog allergens, endotoxin or $\beta(1,3)$ -glucans] were associated with allergic sensitization (to any allergen or to inhalant allergens) at 10 years of age. At age 10, 10.4% and 13.9% of the children were sensitized to cat and dog allergens, respectively. However, no significant associations were found between cat and dog allergen exposure at age 2 and sensitization to the respective allergens at 10 years of age in univariate or multivariate models. Of the 36 children sensitized to dog, all but three (91%) had *never* kept a dog at home, and 22/26 (85%) cat-sensitized children had never had a cat at home. Moreover, at 10 years of age, only 8/48 (16.7%) and 7/27 (25.9%) families with cats or dogs, respectively, had kept a cat or a dog continuously since the child's birth.

Cat and dog allergen levels at 2 years and asthma, bronchial hyperresponsiveness and lung function at 10 years

The risk of current asthma at age 10 increased with an OR of 1.20 [95% CI: 1.01–1.43], $P = 0.04$ per $10 \mu\text{g}$ increase in cat allergen exposure at home at 2 years of age. This association is illustrated by the predictive probability of asthma for boys and girls in Fig. 2. However, when stratified by allergic sensitization, the association between cat allergen exposure at age 2 and current asthma at age 10 was significant only for children *without* allergic sensitization [aOR 1.32 (1.07–1.62), $P = 0.009$]. No association was seen for dog allergen levels at 2 years and the risk of asthma at 10 years of age.

The risk of BHR at age 10 years increased with 1.22 (1.02–1.46), $P = 0.03$, per $10 \mu\text{g}$ increase in cat allergen exposure at the age of 2 years. The relationship between cat allergen exposure and BHR was significant even when an adjustment was made for current asthma [aOR 1.20 (1.00–1.44), $P = 0.05$]. No association was seen between dog allergen levels and the risk of BHR at 10 years of age.

Cat and dog allergen concentrations at 2 years were not associated with FEV_1 or FEF_{50} % predicted, or with a change in lung function from birth to 10 years of age.

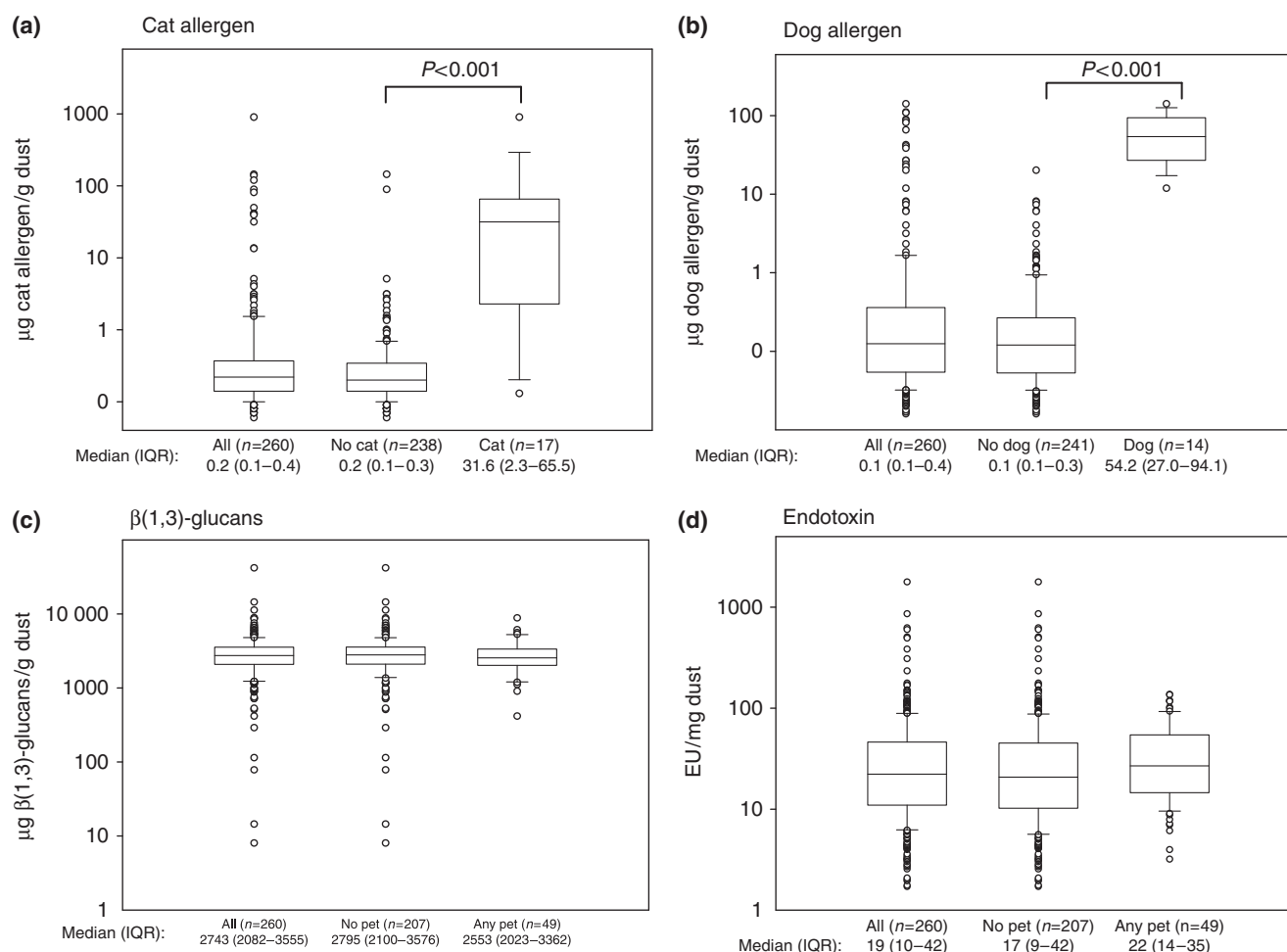


Fig. 1. The graphs show the distribution of the (a) cat allergen, (b) dog allergen, (c) $\beta(1,3)$ glucans and (d) endotoxin as box plots. The boundaries of each box represent the 25th and 75th percentile (interquartile range), and the line within the box marks the median (values are given below the x-axis). The error bars below and above the box indicate the 10th and the 90th percentiles. The outlying points are marked as dots. Note logarithmic scales on the y-axis. The first box plot in each figure shows the distribution for the dust samples from all the 260 children. The second box plot is for homes without pets and the third for homes with pets.

Endotoxin and $\beta(1,3)$ -glucan exposure

The FEV_1 (% predicted) decreased with 0.12 [regression coefficient (b), 95% CI: (–0.20 to –0.03), $P=0.01$] per 10 EU/g dust increase in endotoxin exposure in a multivariate linear regression model. However, the association was no longer significant after exclusion of one extreme outlier in the endotoxin concentration.

Exposure to endotoxin or $\beta(1,3)$ -glucan concentration at 2 years did not modify the risk of any of the other health outcomes at 10 years of age.

Discussion

In the present study with a low community prevalence of pet keeping during the 2 first years of life, exposure to cat allergen at 2 years increased the risk of current asthma at 10 years of age, but did not increase the risk of allergic

sensitization at age 10. Neither endotoxin, $\beta(1,3)$ -glucan nor dog allergen exposure at age 2 modified the risk of allergic disease at age 10.

The increased risk of asthma associated with early life exposure to cat allergen is in line with the conclusions from a systematic review where exposure to pets early in life appeared to increase the risk of asthma in children after the age of 6 [16]. In contrast, a meta-analysis found exposure to dogs to be a risk factor for the development of asthma, whereas exposure to cats appeared to be protective. The frequency of pet keeping appeared to be an important effect modifier, with exposure to pets as a strong risk factor only in countries where pet keeping at home was uncommon [38]. The prevalence of pet keeping was low in the present cohort compared with the majority of other European birth cohorts [39]. Several factors may underlie the inconsistencies between the study results. First the systematic review [16] did not distinguish

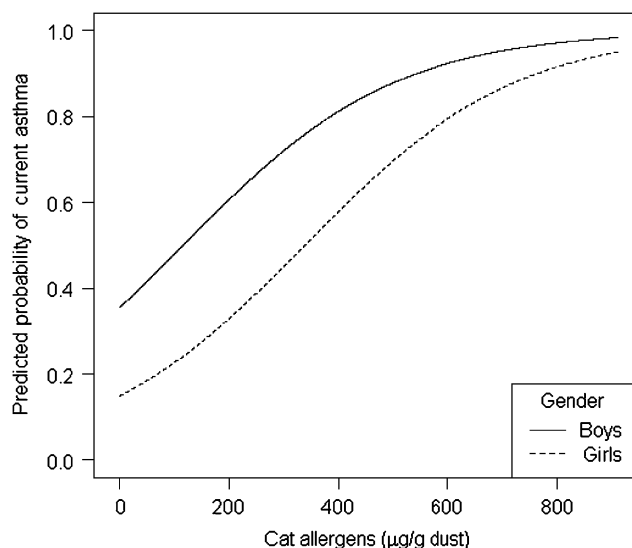


Fig. 2. The predicted probability of current asthma at age 10 among boys and girls based on risk calculation [odds ratio (OR)] per increase in cat allergen exposure at age 2, adjusted for alcohol in pregnancy (no), parental rhinoconjunctivitis at birth (no) and parental education (category 4: college, vocational).

between different types of pets, and secondly, the conclusions from the meta-analysis [38] did not take into account whether the included studies assessed exposure to cat allergens or cat keeping [40]. Pet allergen exposure may depend on the community prevalence of furry pets [17–19]. In particular, pet allergens contaminate schools and homes without pets due to transfer of allergens on clothes [18, 19, 41]. Furthermore, pet avoidance might confound associations between pet allergen exposure or pet keeping and allergic diseases [39]. However, in the present study, pet ownership at the child's birth did not differ between the families with and without a history of parental allergic disease [42].

Exposure to allergens and microbial agents early in life when the lungs are still under development may have consequences for lung function and development of asthma later in childhood [24, 25]. This was demonstrated in the German MAS study, where children who were sensitized and exposed to cat and mite allergens before, but not after, 3 years of age had reduced lung function at age seven [9]. Except for the present study, we are not aware of other birth cohort studies that have reported associations with early life cat allergen exposure and asthma in children after the age of 6 years [11–13]. Although birth cohort studies are likely to give the best answers regarding the risks associated with allergen exposure in childhood, most tend to have short follow-up times and it may still take a few more years before we can obtain more final answers from these studies [16, 43].

In the present study, the effect of exposure to cat allergens was consistent for *non-allergic* current asthma only and no association was seen with allergic sensitiza-

tion. The relationship between early life allergen exposure and allergic sensitization in other cohort studies is inconsistent, with an increased risk of sensitization to cat in early childhood [13, 44, 45], but not in older children [44, 46]. Furthermore, early life exposure to mite allergens has been associated with mite sensitization [13] particularly in high-risk children [47] whereas others reported no such associations [11]. Moreover, in areas with a high exposure to mite allergen and a correspondingly high prevalence of mite-sensitized individuals, a tolerance induction for sensitization against cats has been demonstrated for those exposed to high concentrations of cat allergens [7]. In the present study, sensitization to mites (8.0%) and moulds (2.8%) was low compared with other countries reporting 39–44% for mite sensitization [7, 48] and 10% for moulds [48]. Furthermore, cat and dog allergens were the most common sensitizing allergens (10.4% and 13.9%, respectively) among the indoor allergens tested, although as many as 85% of the children with sensitization to cat allergens had never had a cat at home (and 91% for dog). This is in line with reports from Northern Sweden, where 83% of the children with cat sensitization had never lived with a cat [49]. These results are in agreement with the notion that in communities with low mite allergen exposure and a low prevalence of cat and dog ownership, indirect exposure to cat and dog allergens may be more important for allergic sensitization than keeping the pets at home [18].

In contrast to other birth cohort studies [14, 21, 50], we found no evidence of modifying effects by endotoxin or $\beta(1,3)$ -glucan levels in house dust. The possible decrease in FEV₁ % predicted at 10 years of age was uncertain, because it was no longer significant after excluding one subject with extremely high endotoxin concentrations. In at least one of the previous studies, the children had been exposed to much higher levels of endotoxins (lowest quartiles: 2.1–52.5 and 126–713 EU/mg in the highest quartile) [14] than in the present study [median (IQR): 19(10–42) EU/mg]. However, a more plausible explanation for the lack of association in the present study compared with these three other birth cohort studies may be that they were all performed on high-risk children [14, 21, 50]. The co-variation between the environmental compounds can be a potential confounder in studies of environmental compound exposures, such as demonstrated for mite and pet allergens and endotoxin [51]. In the present study, only poor correlations were found between the environmental compounds and all compounds were mutually adjusted for each other by initially being included in the same model.

Strengths and limitations

The present analyses of risk factors were based on a large number of factors assessed at birth that were known to

increase the risk of allergic disease at age 10 for the children in this particular birth cohort, including lung function measurement at birth [23], which is unique for the present cohort. Although only 260 children were included in this study, the reported risk association of asthma at 10 years with cat allergen exposure at 2 years represents valuable information also because of the long follow-up time between exposure assessment and outcome.

Comparing dust samples from different studies is difficult due to differences in sampling, extraction and storage procedures. Most studies have dust samples from mattresses or a limited area of the floor, which are both different from the present study, where the samples represent an overall exposure of the living area of the house (living room, kitchen and bedrooms). It has been argued that the latter may be a better surrogate marker for assessment of a child's overall exposure in the home than mattress dust, as it also reflects exposure to additional allergen sources such as furniture and toys [12]. It was only possible to measure allergen concentrations ($\mu\text{g/g}$ dust) – and not the absolute amount of allergens ($\mu\text{g/m}^2$). However, absolute allergen content reported per m^2 is highly dependent on the dust-collecting procedure, and therefore is not preferred in settings like ours, where the parents themselves collected the dust.

It is unclear what effect, if any, the storage time had on the measured levels of allergens and microbial agents. However, it has been shown that at least for endotoxin, only freeze–thaw cycles and not the duration of storage affected endotoxin activity [52]. Levels of mite allergens, endotoxin and $\beta(1,3)$ -glucan in dust samples were stable at -20°C stored for up to 10 months (before extraction) in one study, whereas Fel d1 concentrations declined with increasing storage time [53]. In the present study, all the samples were treated equally, and not subjected to freeze–thaw cycles. Thus, a potential decrease in the measured concentration levels would presumably affect all samples equally. The low levels of mite allergens are in line with previous findings from other sites in Scandinavia [18, 54], as well as from mattress dust in a nested case–control study within the present cohort [55].

In summary, the present study demonstrated that exposure to high cat allergen levels at home in early childhood increased the likelihood of current asthma as well as BHR at 10 years, whereas no association was found with allergic sensitization. Endotoxin, $\beta(1,3)$ -glucan and dog allergen exposure at age 2 did not modify the risk of asthma, allergic sensitization and lung function at 10 years of age.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Appendix S1. Childhood asthma and early life exposure to indoor allergens, endotoxin and $\beta(1,3)$ -glucans.

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