

Associations between selected allergens, phthalates, nicotine, polycyclic aromatic hydrocarbons, and bedroom ventilation and clinically confirmed asthma, rhinoconjunctivitis, and atopic dermatitis in preschool children

Abstract Previous studies, often using data from questionnaires, have reported associations between various characteristics of indoor environments and allergic disease. The aim of this study has been to investigate possible associations between objectively assessed indoor environmental factors and clinically confirmed asthma, rhinoconjunctivitis, and atopic dermatitis. The study is a cross-sectional case–control study of 500 children aged 3–5 years from Odense, Denmark. The 200 cases had at least two parentally reported allergic diseases, while the 300 controls were randomly selected from 2835 participating families. A single physician conducted clinical examinations of all 500 children. Children from the initially random control group with clinically confirmed allergic disease were subsequently excluded from the control group and admitted in the case group, leaving 242 in the healthy control group. For most children, specific IgE's against various allergens were determined. In parallel, dust samples were collected and air change rates were measured in the children's bedrooms. The dust samples were analyzed for phthalate esters, polycyclic aromatic hydrocarbons (PAH), nicotine, and various allergens. Among children diagnosed with asthma, concentrations of nicotine were higher ($P < 0.05$) and cat allergens were lower ($P < 0.05$) compared with the healthy controls; air change rates were lower for those sensitized (specific IgE+) compared with those not sensitized (specific IgE–, $P < 0.05$); and dust mite allergens were higher for specific IgE+ cases compared with healthy controls ($P < 0.05$). When disease status was based solely on questionnaire responses (as opposed to physician diagnosis), significant associations were found between di(2-ethylhexyl) phthalate (DEHP) and dog allergens in dust and current wheeze.

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Practical Implications

The associations observed in this study reinforce the value of recommending adequate ventilation and avoidance of environmental tobacco smoke (ETS) when providing guidance to families of children with allergic disease. The findings regarding phthalate esters are less conclusive (perhaps because of lower phthalate levels in this study compared with previous ones). The association between DEHP and children's wheeze, as reported by parents in the questionnaire, is significant and matches findings from earlier investigations. Not surprisingly, a detailed examination by a physician results in a different diagnosis of allergic disease than is obtained from a questionnaire that is filled out by a parent. Presumably, the former is preferable in studies probing potential associations between indoor environmental factors and allergic disease in early childhood.

Introduction

For decades, associations have been made between various environmental factors and allergic disease. However, showing a direct relation between factors in the indoor environment and upper airway diseases like asthma and allergic rhinoconjunctivitis is not straightforward (Asher et al., 2004; Halken, 2004; Host et al., 2008; Institute of Medicine, U.S., 2004). A prospective study with continuous measurements and clinical evaluations starting with a birth cohort might be the best way to study potential associations, but it would be very costly and difficult to actually execute such a study. Current knowledge is primarily derived from cross-sectional studies with the concomitant limitations of such an approach. Additionally, conclusions have often been based on questionnaire reports (Heinrich, 2011), which may not be as reliable as measurements of environmental parameters coupled with physician assessment of disease. These considerations emphasize the importance of carrying out investigations with solid endpoints to improve the likelihood that such studies are useful (Tovey and Marks, 1999).

Indoor exposures to pollutants that may impact allergic disease are varied and abundant for preschool children. Associations between house dust mite allergens and pet (cat and dog) allergens and asthma have been extensively studied (Chen et al., 2010; Gold, 2000; Harving and Korsgaard, 1994; Heinrich, 2011). While these allergens can promote symptoms in children who have already developed asthma, the evidence is less conclusive regarding the role of these allergens in the development of asthma (Gold, 2000; Heinrich, 2011). In the case of cat allergens, the results are even more confusing. Some studies indicate that early exposure to cat allergen results in sensitization [i.e., a positive IgE response (Brussee et al., 2005; Heissenhuber et al., 2003)]. Other studies suggest that early exposure to cat allergen helps to develop the immune system [i.e., the hygiene hypothesis (Custovic et al., 2001; Platts-Mills et al., 2001)].

Øie et al. (1997) suggested that phthalates might be contributing to increases in allergies and asthma among children in developed countries. As reviewed by Jaakkola and Knight (2008), a number of studies have since reported associations between children's exposure to phthalate esters or PVC materials, presumably plasticized with phthalates, and allergic disease (e.g., Bornehag et al., 2004; Hsu et al., 2012; Jaakkola et al., 1999; Jaakkola et al., 2000; Jaakkola et al., 2004; Kolarik et al., 2008; Øie et al., 1999). Of these studies, those by Bornehag et al. (2004) and Kolarik et al. (2008) are closest in design to this study. Bornehag et al. used a case-control study ($n = 400$) of children from Varmland, Sweden, to examine potential associations between the concentration of phthalate esters in dust collected from the children's bedrooms and allergic symptoms. They found an association between

concentrations of butyl benzyl phthalate (BBzP) in dust and rhinitis ($P = 0.001$) and eczema ($P = 0.001$), and between di(2-ethylhexyl) phthalate (DEHP) in dust and asthma ($P = 0.022$). Kolarik et al. (2008) conducted an analogous case-control study ($n = 272$) of children from Sofia and Burgas, Bulgaria. They found higher concentrations of DEHP in the bedroom dust of cases compared with controls (1.24 vs. 0.86 mg/g dust) and that DEHP in bedroom dust was associated with parent-reported wheezing in their children during the preceding 12 months ($P = 0.035$).

Polycyclic aromatic hydrocarbons (PAHs) are common products of incomplete combustion. They are major constituents of motor vehicle exhaust and can be used as markers for exposure to traffic-derived pollution (Oda et al., 2001). Exposure to traffic pollutants has been linked to asthma and allergic diseases (Jerrett et al., 2008; McConnell et al., 2006; McCreanor et al., 2007; Salam et al., 2008; Zmirou et al., 2004). More specifically, traffic-derived pollution has been associated with the development of asthma and allergies during the first 8 years of life (Gehring et al., 2010).

Nicotine in settled dust can be used as a marker of environmental tobacco smoke (ETS) exposure (Kim et al., 2008; Whitehead et al., 2009). Children's exposure to ETS adversely impacts their lungs and increases their risk of asthma (Cook and Strachan, 1997; Heinrich, 2011; Pietinalho et al., 2009). Indeed, there is a 'strong and consistent association between ETS and asthma development in young children' (Gold, 2000).

As is apparent from the preceding paragraphs, for certain indoor environmental factors, associations with allergic disease/asthma are less well established than for others. In part, this reflects limitations inherent in the design of a number of previous studies. The aim of this study has been to further investigate associations between selected indoor environmental factors and asthma, allergic rhinoconjunctivitis, and atopic dermatitis among approximately 500 preschool children from the city of Odense, DK. In this study, we use well-defined diagnostic criteria, clinically confirmed in a detailed examination of each child by a physician, coupled with determination of sensitization (specific IgE) to define a child's allergic disease status. The environmental factors included in this investigation are house dust mite and pet allergens in bed dust; mass fractions of phthalate esters, nicotine, and PAHs in settled dust from the children's bedrooms and day care centers; and air change rates in the child's bedroom.

Material and methods

Subjects

This study is a part of the Indoor Environment and Children's Health study (IECH), which is summarized in the study of Clausen et al. (2012). Figure 1 presents

a flowchart outlining the different phases of IECH. The first phase of the study included a written questionnaire (WQ) with 116 questions regarding building/indoor environment characteristics, family habits, and health with a focus on allergic diseases (asthma, rhinoconjunctivitis, and atopic dermatitis). The WQ was mailed to 17,500 families living in Funen with a child aged 1–5 years in February 2008. The response rate was 63%, corresponding to 11,084 families. From these, we selected 2835 families with children aged 3–5 living in Odense (main city on the island of Funen; population 166,000) or one of its suburbs or rural neighborhoods. For this study, we chose a case-control design. Based on power calculations, 500 total subjects were selected to be part of the case-control group. Controls were initially 300 randomly selected children (random controls) representing a sample of the general population, including both children with and without allergic diseases. The selection criteria for the 200 children in the case group were at least two parentally reported disease/symptoms regarding asthma, allergic rhinoconjunctivitis, or eczema/atopic dermatitis on the WQ (Callesen, M., Jensen, T.K., Norberg, L.A., Clausen, G., Toftum, J., Bekö, G., Sundell, J., Bornehag, C.-G., Sigsgaard, T. and Høst, A., submitted).

Clinical examination. All children underwent a clinical examination and interview (CEI) by the same experienced medical doctor. Weight and height were recorded, and an extensive questionnaire-based interview, including medical history, time and onset of symptoms, trigger factors, allergic heredity, and exposure to pets and tobacco smoke, was conducted. The medical history was supplemented with the use of a video exemplifying asthma in 3–5-year-olds and a demonstration of sounds indicative of asthma.

The following diagnostic criteria, based on internationally accepted guidelines (Bousquet et al., 2008; Hanifin and Rajka, 1980; Johnke et al., 2005; Niggemann et al., 2006; Rothe and Grant-Kels, 1996; Soto-Martinez et al., 2009), had to be met for a child to be labeled with one of the allergic diseases during the clinical examination:

Asthma was diagnosed when one of the following internationally accepted criteria was met (Muraro et al., 2004; Soto-Martinez et al., 2009): (i) recurrence of at least two of the three symptoms: cough, wheeze, and shortness of breath within the previous 12 months (symptoms other than those triggered by respiratory infections); and (ii) doctor's

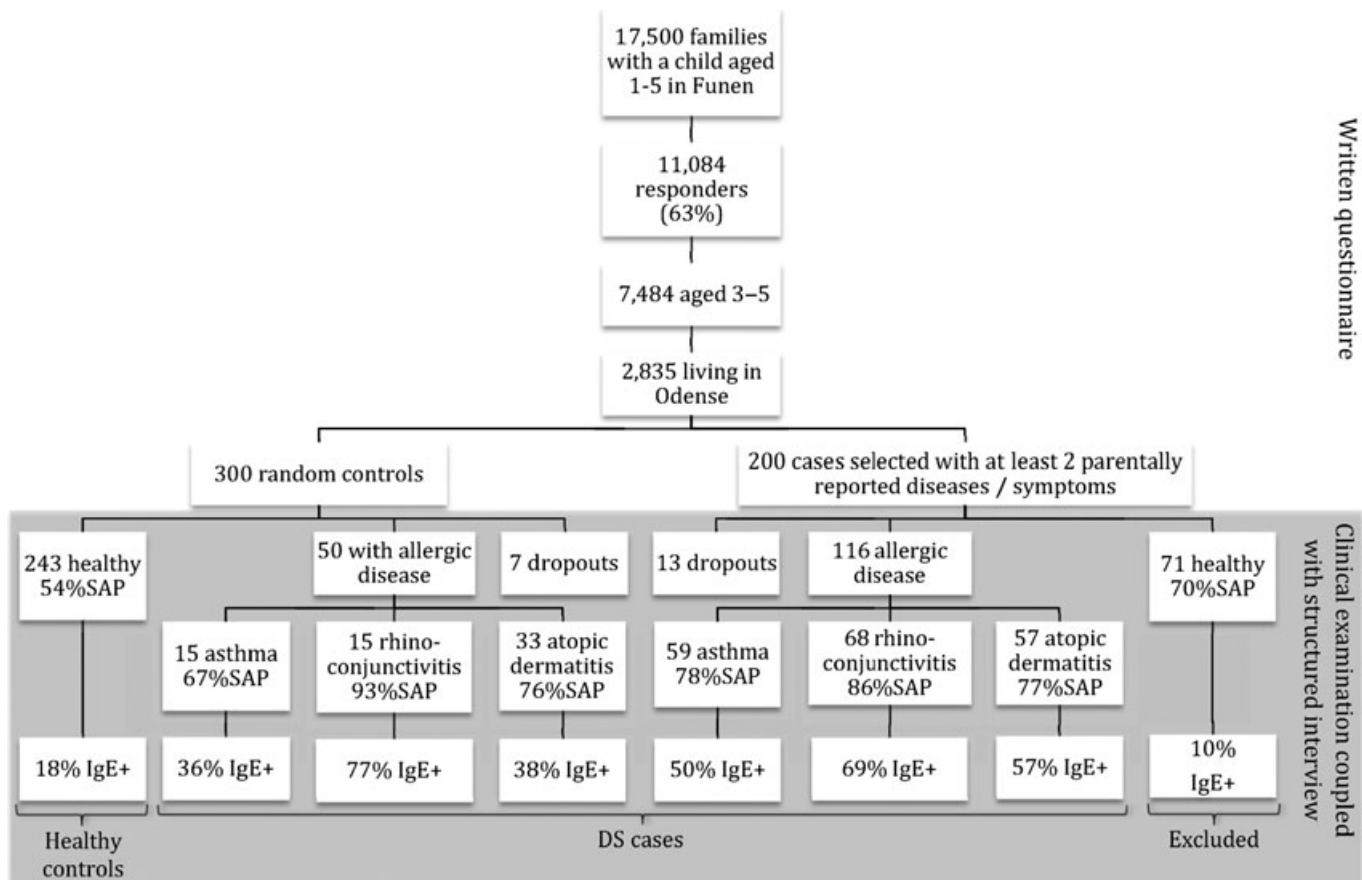


Fig. 1 Flowchart for the Danish Indoor Environment and Children's Health study. In the analyses, one child was excluded from the healthy control group ($n = 242$) due to incomplete data. The subgroups under the allergic disease categories are not mutually exclusive. SAP, single allergic predisposition. IgE+, specific IgE sensitization

diagnosis of asthma in combination with ongoing treatment. Further, a clear response to treatment with β_2 -agonists and/or inhaled corticosteroids was demanded for the diagnosis (Niggemann et al., 2006; Soto-Martinez et al., 2009). Children with no clear-cut diagnosis but with symptoms suggestive of asthma such as isolated persistent cough, wheeze, or shortness of breath, exercise-induced symptoms or reduced physical activity were subsequently evaluated by an expert on pediatric asthma and diagnosed as asthmatics ($n = 5$) in the instance of response to asthma medication.

Allergic rhinoconjunctivitis was diagnosed according to internationally accepted criteria in children with recurrence of at least two or more symptoms from the nose (pruritus, runny nose, sneezing spells > 20 , nasal stenosis/mouth breathing) and eyes (itching, conjunctival injection, or watery secretion in both eyes; Bousquet et al., 2008) when exposed to relevant allergens. Allergic rhinoconjunctivitis was used as an umbrella term for allergic conjunctivitis, allergic rhinitis, or both.

Atopic dermatitis was diagnosed according to criteria established by Hanifin and Rajka (1980) requiring the presence of at least three of four major features and at least three of 23 minor features. Five of the 23 minor criteria were excluded (skin prick test, keratoconus, anterior sub-capsular cataract (as this required an ophthalmologist), early age of onset white dermographism (because of age of study population), and delayed blanch; Hanifin and Rajka, 1980; Johnke et al., 2005; Rothe and Grant-Kels, 1996).

IgE measurements. Specific IgE was measured by ImmunoCap (Phadia, Allerød, Denmark) for a total of 20 allergens: (i) indoor allergens (*Dermatophagoides pteronyssinus*, *Dermatophagoides farinae*, cat, horse, and dog); (ii) outdoor allergens (grass, common silver birch, mugwort, *Penicillium chrysogenum*, *Cladosporium herbarum*, *Aspergillus fumigatus*, *Candida albicans*, *Alternaria alternata*, and *Setomelanomma rostrata*); and (iii) food allergens (egg white, milk, fish, wheat, peanut, and soybean). Specific IgE was classified as positive when higher than 0.35 kilo units of antibody per liter (kU/l; Bousquet et al., 2008; Eigenmann et al., 2013).

Indoor environment

Detailed investigations of the homes and day care facilities were conducted by trained inspectors (students and employees from the International Centre for Indoor Environment and Energy at the Technical University of Denmark) who visited all 500 homes and 151 day care centers between March and May, 2008.

Visits to cases and random controls were relatively evenly distributed throughout the 10 week-campaign.

Allergens in dust. Dust was sampled from children's beds. Samples were sieved, weighed, and a soluble extract prepared using a sequential protocol described by Spaan et al. (2008). The concentrations in the extract of allergens from house dust mite (Der p1, Der f1, and Der p2), cat (Fel d1), and dog (Can f1) were measured using fluorescent 5-plex MARIA assay (Indoor Biotechnologies, Charlottesville, VA, USA; Earle et al., 2007). Briefly, the extraction of the samples was performed in 5 ml pyrogen-free water with 0.05% (v/v) Tween-20. The samples were initially shaken for 60 min on a Multi Reax digital shaker (Heidolph Instruments GmbH, Schwabach, Germany) and then centrifuged for 15 min at 218 g. Subsequently, 1 ml of the supernatant was removed, aliquoted in four 0.1 ml portions, and stored at -20°C . The analyses were performed in duplicate. The assays' limit of detection (LOD) was 0.06 ng/ml, and the LOD for allergen concentrations in dust was 12 ng/g. Values below the limit of detection were replaced with 2/3 of the LOD.

Phthalates, PAHs, and nicotine in dust. Dust samples were taken from horizontal surfaces above floor level in the child's bedrooms ($n = 500$), as well as from the day care centers they attended ($n = 151$). The compounds targeted for analysis in these samples included five phthalate esters: diethyl phthalate (DEP), di(n-butyl) phthalate (DnBP), di(isobutyl) phthalate (DiBP), butyl benzyl phthalate (BBzP), and di(2-ethylhexyl) phthalate (DEHP); three PAHs: pyrene, benzo[a]anthracene (BAA), and benzo[a]pyrene (BAP); and nicotine. The method used to collect the dust samples and the analytical procedures used to measure the mass fractions of the targeted compounds in these samples have been described in detail in an earlier paper (Langer et al., 2010). In brief, the dust was vacuumed from nonfloor surfaces in the children's bedrooms or day care centers onto a cellulose-filter (ALK-Abello A/S, Hørsholm, Denmark) mounted in a holder connected to a vacuum cleaner hose. In 2% of homes, a duplicate sample was collected, and in 5% of the homes, a field blank was collected. The amount of dust on the loaded filters ranged from 40 to 1400 mg. At the beginning of the chemical analysis, the dust samples were spiked with isotopically labeled internal standards (diethyl phthalate- d_4 , di-n-octyl phthalate- d_4 , chrysene- d_{12} , and benzo[a]pyrene- d_{12}). They were then ultrasonically extracted twice using n-hexane mixed with 6% diethyl ether. The two extracts were combined and concentrated to approximately 5 ml. A 250 μl aliquot of the concentrated extract was analyzed for the phthalate esters and nicotine after adding the injection standard (1,2,3,4-tetrachloronaphthalene). Following the phthalate and nicotine analysis, the extracts were further

concentrated and cleaned on a solid phase column (IST Isolute, part nr. 910-0100-B). The fraction containing the PAHs was dried, reconstituted with 250 ml of n-hexane, and spiked with the injection standard prior to analysis. Analyses for all of the targeted compounds were performed on an Agilent Technologies (Santa Clara, CA, USA) 6890N Gas Chromatograph equipped with a 5975 XL Mass Selective Detector (GC-MS) operating in full scan acquisition mode (29 – 650 amu). The capillary column was BPX-5 (5% phenyl polysilphenylene siloxane), 25 m, 0.22 mm i.d., 0.25 μ m film thickness. The carrier gas was ultrahigh purity helium. The recoveries of the deuterated internal standards were 90% or larger in all samples and blanks. Values below the limit of detection were replaced with 1/2 of the LOD.

Measurements of ventilation rate. The concentration of carbon dioxide was continuously measured over a minimum of 2 days and two nights in the bedrooms of the children. CARBOCAP[®] CO₂ monitors (GMW22, Vaisala, Finland) measuring units, connected to a data logger, were used to measure the CO₂ concentration. The measuring interval was 5 min. To estimate the CO₂ emission rate in the room during the period of the measurements, the parents were asked to record the number of occupants in the room for each day and each night. They were also asked to record if anyone left the room for an extended period during the night. They provided the weight and height of each person sleeping in the room. Additionally, the volumes of the measured rooms were measured. Carbon dioxide data obtained in the time period between 21:00 and 7:00 each night of measurement were extracted for the purpose of calculating the ventilation rate. Data from periods when the child was not in the room were omitted. Primarily, periods of readily discernible concentration build-ups were used to determine the ventilation rate in the room for the night in question. The air change rate in the room was estimated by fitting a curve to the measured pattern of the CO₂ concentration at a given CO₂ emission rate, room volume, and outdoor CO₂ concentration. The final ventilation rate in a bedroom was the time-weighted average of the ventilation rates obtained for each relevant time period. More detailed descriptions of the measurements, the ventilation rate calculations, and the associated limitations have been published in a previous paper (Bekö et al., 2010).

Statistical method

Children with clinically confirmed allergic disease were subsequently excluded from the *random control group* (and admitted in the case group) leaving 243 children in the *healthy control group*. Due to incomplete data for one child, analyses were performed on 242 healthy controls. Children with asthma, allergic rhinoconjunc-

tivitis, or atopic dermatitis based on the CEI (disease-specific cases, referred to as DS cases in what follows) were compared with the healthy control group subjects to investigate potential associations between the health outcomes and any of the five allergens measured in bed dust, air change rates, and mass fractions of phthalates, PAHs, and nicotine measured in settled dust from the homes and day care centers (DCC). In these analyses, children in the original case group ($n = 200$) without clinically confirmed allergic disease were excluded ($n = 71$). The DS case groups were also divided into specific IgE-positive and specific IgE-negative children and compared with the healthy control group. Additionally, doctor-diagnosed allergic diseases and current wheeze, as reported by the parents using the written questionnaires, were used to obtain four new DS case groups. Only children that did not belong to the healthy control group were used to create these groups (500 – 243 healthy controls – 20 dropouts = 237 children). The analyses approach used to compare these groups with the healthy controls were identical to the analyses used for the DS case groups determined from the CEI.

Dust allergens, air change rates, and dust mass fractions of phthalates and PAHs were log-normally distributed. For comparisons of medians for healthy controls and the various DS case groups, the Mann–Whitney *U*-test was used. Multiple logistic regressions were performed after factoring the allergens, air change rates, and phthalates into quartiles, thirds, and high/low depending on the number of subjects. We set a limit of at least 17 subjects in each subgroup, to perform the respective analyses. We also performed linear regression for the log-normally distributed variables that did not have values below the limit of detection (e.g., DEHP mass fractions). Both crude and adjusted analyses were performed. The analyses were adjusted for allergic predisposition, gender, breastfeeding less than 3 months, smoking inside the home, air change rates, and phthalate mass fractions.

When analyzing the association between phthalates in the dust and DS case status, we used phthalate concentrations in the dust from both the children's homes and their day care facilities. We also estimated the total phthalate exposure of the children within the two environments, expressed as a weighted phthalate mass fraction. We assumed that a child spends 20 h a day indoors in one of the two environments (140 h indoors per week; we assume 4 h a day spent outdoors, in transit and in other indoor environments). From this time period, we assumed 6 h to be spent indoors in the DCC (30 h per week) and 14 h indoors at home during weekdays. During weekends, we assumed that a child spends all 20 h per day indoors at home (110 h per week). Thus, the weighted dust mass fraction used in the analyses of the association between phthalates

and DS case status was calculated as follows:
 $C_{\text{dust_combined}} = (3.6 \times C_{\text{dust_home}} + C_{\text{dust_day care}})/4.6$.

STATA software, release 11.2 (StataCorp LP, College Station, TX, USA), was used for statistical analysis. The study was approved by The Regional Scientific Ethical Committee for Southern Denmark (Case # S-20070108).

Results

Figure 1 summarizes the selection of the 500 children and subsequent categorization based on their health status as determined by the CEI. In the group of 300 random controls, asthma was diagnosed in 5.1% of the children, allergic rhinoconjunctivitis in 5.1%, and atopic dermatitis in 11.3% by CEI. Seven families (2.3%) dropped out because they did not want to participate, moved outside the country, or could not be contacted. In the group of 200 cases selected based on at least two parentally reported allergic diseases, 38% ($n = 71$) did not fulfill the criteria for any allergic disease based on the physician's CEI and 13 subjects dropped out prior to the CEI. Thirty-six percent of the random controls and 30% of the cases refused to give a blood sample. (Figure 1 also shows the percent of subjects in different categories that have at least one parent with allergic disease – 'single allergic predisposition (SAP)'. Although this information is not further discussed in the paper, it is included for future reference.) Being diagnosed with more than one allergic disease by the CEI was more common among the IgE-sensitized cases (Figure 2). Table 1 presents median concentrations of indoor allergens measured in the dust collected from the child's bed, median air change rates, and the concentrations of nicotine and PAHs in the dust collected from the home. These results are presented for the healthy control group ($n = 242$, mean age 4.4) and the three DS case groups: asthma case group ($n = 74$, mean age 4.6), rhinoconjunctivitis case group ($n = 83$, mean age 4.7), and atopic dermatitis case group ($n = 90$, mean age 4.6) as determined by the CEI. Table S1 presents results for the identical tests performed on

the healthy controls and four DS case groups based on the written questionnaire (doctor-diagnosed asthma, allergic rhinoconjunctivitis, and atopic dermatitis; and current wheeze). In what follows, we will primarily focus on results of the analyses performed on the DS case groups that were based on the CEI.

Asthma case group

A tendency for higher concentrations of dust mite allergen in the dust samples was observed for the asthma cases, compared with the healthy controls. The concentrations of dust mite allergens Der f1 and Der p2 were lower among the non-sensitized (specific IgE negative) children compared with the healthy control group and higher among the sensitized (specific IgE positive) children compared with the healthy control group ($P < 0.05$ for Der f1). We found a significantly lower concentration of cat allergen Fel d1 and dog allergen Can f1 in the asthma group compared with the healthy controls. Significantly lower cat allergen levels were also observed among the IgE-positive asthma cases when compared with the healthy controls or to the IgE-negative asthma cases.

The asthma children had slightly higher air change rates in their bedrooms than the healthy controls. In contrast, air change rates were significantly higher in the IgE-negative asthma group than in the IgE-positive asthma group (Figure 3). Median mass fraction of nicotine in the dust was significantly higher for asthma cases than for healthy controls.

Rhinoconjunctivitis case group and atopic dermatitis case group

House dust mite allergen concentrations were higher, while cat and dog allergen concentrations were lower in the rhinoconjunctivitis case group compared with the healthy control group. Lower allergen levels were measured in the homes of the IgE-negative rhinoconjunctivitis group compared with the IgE-positive rhinoconjunctivitis group, with the exception of Der p1 and Der p2 dust mite allergens (the differences were not significant). No significant differences were found in the concentrations of nicotine and PAHs between the children with allergic rhinoconjunctivitis and the healthy controls.

No significant differences were observed between (i) either the atopic dermatitis case group and the healthy control group; or (ii) between the IgE-negative and IgE-positive atopic dermatitis cases with respect to allergen concentrations, air change rates, and concentrations of nicotine and PAHs in the dust.

Pets and cleaning habits

Ten percent of all families owned a dog. The percentage of families with cats are shown in Table 2 broken down

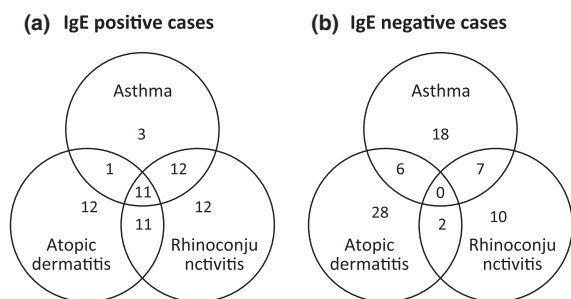


Fig. 2 Occurrence of allergic diseases among the specific IgE-positive (a) and specific IgE-negative (b) disease-specific cases (i.e., DS cases based on the clinical examination and interview)

Table 1 Dust allergen concentrations, air change rates, bedroom volume and mass fractions of nicotine and polycyclic aromatic hydrocarbons in the dust (median values) in the bedrooms of 242 randomly selected children who were clinically confirmed to be without allergic disease (healthy controls) compared with children with asthma, allergic rhinoconjunctivitis, or atopic dermatitis as determined from a clinical examination

	N	Der p1 (ng/g)	Der f1 (ng/g)	Der p2 (ng/g)	Fel d1 (ng/g)	Can f1 (ng/g)	ACH	Bedroom vol (m ³)	Nicotine (μg/g)	Pyrene (μg/g)	BAA (μg/g)	BAP (μg/g)
Healthy controls ^c	242	110.0	977.2	121.5	114.5	81.6	0.39	25.1	0.87	0.12	0.017	0.009
Asthma ^c	74	132.2	988.4	119.5	76.7***	71.1*	0.50	22.6	1.58**	0.13	0.019	0.008
s-IgE negative	31 ^b	145.4	471.8	69.2	201.7	77.7***	0.56	22.0	2.89***	0.12	0.017	0.007
s-IgE positive	27 ^b	132.0	1726***	135.5	42.1*** ^a	71.8	0.41 ^a	23.5	1.50	0.14	0.022	0.010
Rhinoconjunctivitis ^c	83	119.4	1251	153.4	85.6	54.2	0.47	23.5	0.09	0.13	0.016	0.009
s-IgE negative	19 ^b	164.9	173.7	158.1	61.6	28.5	0.37	22.4	1.10	0.14	0.015	0.010
s-IgE positive	45 ^b	104.8	988.4*	130.4	99.6	66.4	0.43	26.6	0.09	0.12	0.016	0.009
Atopic dermatitis ^c	90	131.8	751.9	119.5	150.4	53.0	0.48	25.0	1.05	0.14	0.020	0.009
s-IgE negative	36 ^b	176.0	672.4	88.3	170.9	53.3	0.50	28.5	0.83	0.14	0.020	0.008
s-IgE positive	34 ^b	151.0	1211	130.0	116.6	57.2	0.43	23.5	1.22	0.14	0.019	0.010

Der p1, Der f1, Der p2, dust mite allergens; Fel d1, cat allergen; Can f1, dog allergen; ACH, air change rates per hour; BAA, Benzo(a)-anthracene; BAP, Benzo(a)-pyrene; s-IgE, specific Immunoglobulin E.

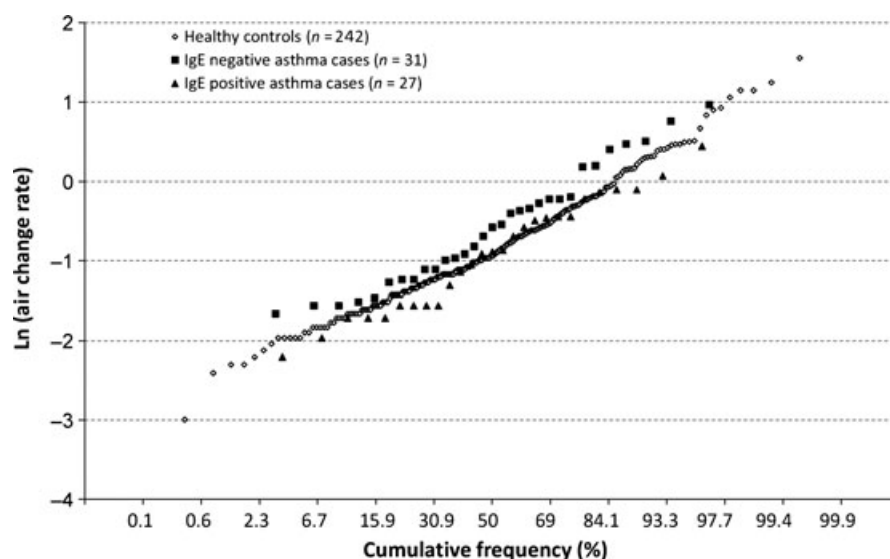
* $P < 0.05$, using Mann–Whitney U -test comparing with the healthy control group.

** $P < 0.05$, using regression models (logistic on high/low, linear), compared with the healthy control group.

^aSignificant difference between specific IgE negative compared with specific IgE positive, in the crude and adjusted linear regression analysis (adjusted for phthalates).

^bNot all cases provided a blood sample.

^cMean age of healthy controls: 4.4; asthma cases: 4.6; rhinoconjunctivitis cases: 4.7; atopic dermatitis cases: 4.6

**Fig. 3** Cumulative frequency distribution of air change rates in the homes of the 242 randomly selected healthy controls, compared with that of the specific IgE-positive and specific IgE-negative children with clinically confirmed asthma

among healthy controls and DS cases. For the healthy controls and DS cases, this table also displays information reported in the WQ regarding pet avoidance behavior, altered cleaning habits due to allergic disease in the family, and bedroom venting and/or airing of the bed duvet within the last 14 days. Pet avoidance was 2.5 times as high in the asthma case group as in the healthy control group. Keeping a cat as a pet was more likely in the healthy control group compared with the asthma case group. However, the IgE-negative asthma case group displayed cat-keeping habits similar to the healthy control group. Families with a child diagnosed with allergic rhinoconjunctivitis were less likely to own

a cat compared with the healthy control group. About 30% of homes with an asthma or rhinoconjunctivitis case changed cleaning habits compared with 6% in the healthy control group. Parent-reported daily bedroom venting and duvet airing were slightly more common among the DS cases than among healthy controls.

Dust phthalate concentrations

Table 3 shows dust mass fractions among healthy controls and various DS case groups selected based on the clinical examination as well as the parentally reported questionnaire data. Associations were examined using

Table 2 Pet-keeping and household habits, as indicated in the written questionnaire, in the healthy control group and in the disease-specific case groups (asthma cases and rhinoconjunctivitis cases based on the clinical examination and interview)

	N	Cat in home (%)	Pet avoidance (%) ^a	Altered cleaning habits (%) ^b	Bedroom venting within the last 14 days			Airing of bed duvet within the last 14 days		
					Daily (%)	≥1 (%)	Not (%)	Daily (%)	≥1 (%)	Not (%)
Healthy controls	242	50 (21)	46 (19)	14 (6)	145 (60)	73 (30)	20 (8)	20 (8)	72 (30)	138 (57)
Asthma	74	9 (12)	40 (54)	24 (32)	48 (65)	13 (18)	7 (9)	9 (12)	19 (26)	41 (55)
Asthma IgE positive ^c	27	1 (4)	17 (63)	9 (33)	17 (63)	8 (30)	0 (0)	6 (22)	7 (26)	12 (44)
Asthma IgE negative ^c	31	7 (23)	13 (42)	8 (26)	20 (65)	5 (16)	5 (16)	2 (7)	9 (29)	19 (61)
Rhinoconjunctivitis	83	12 (14)	42 (57)	25 (34)	53 (72)	19 (26)	5 (7)	15 (20)	27 (36)	34 (46)
Rhinoconjunctivitis IgE positive ^c	46	6 (13)	25 (54)	15 (33)	27 (59)	15 (33)	2 (4)	8 (17)	17 (37)	19 (41)
Rhinoconjunctivitis IgE negative ^c	19	3 (16)	8 (42)	5 (26)	15 (79)	2 (12)	1 (5)	4 (21)	6 (32)	8 (42)

^aGetting rid of or refraining from obtaining a pet due to allergic disease in the family.^bDue to allergic diseases in the family.^cNot all disease-specific cases provided a blood sample.**Table 3** Comparison of phthalate mass fractions in dust (medians, µg/g) from the bedrooms of 242 children in the healthy control group with those from the bedrooms of children in seven disease-specific case groups

	Healthy controls	Clinical examination			Parentally reported questionnaire data			
		Asthma	Rhinoconjunctivitis	Atopic dermatitis	Doctor-diagnosed Asthma	Doctor-diagnosed allergic rhinoconjunctivitis	Doctor-diagnosed atopic dermatitis	Current wheeze ^a
N	242	72	81	88	110	56	83	68
Home								
DEP	1.4	2.5	3.7	1.9	2.5	3.5	2.0	5.4
DnBP	15.1	10.0*	14.1	14.7	13.6	17.2	10.7	9.8
DiBP	27.0	25.8	30.4	33.4	28.9	30.7	26.9	25.6
BBzP	3.9	2.9	3.5	3.9	3.0	2.9	3.2	2.4
DEHP	197	242	247	241	241	243	187	243**
Day care center								
DEP	2.3	1.9	2.1	2.1	2.1	2.1	2.1	2.4
DnBP	35.1	37.5	39.8	39.6	39.2	39.6	36.5	39.6
DiBP	22.6	21.5	22.3	22.5	22.9	23.5	22.4	20.6
BBzP	15.4	18.3	16.6	15.4	18.0	16.6	18.0	15.4
DEHP	485	421	444	452	429	434	452	412
Weighted ^b (home and day care center)								
DEP	1.9	2.8	3.2	2.4	2.9	3.0	2.7	5.5
DnBP	21.7	17.4	22.3	23.1	19.7	23.4	17.5	17.2
DiBP	27.2	25.7	26.8	33.1	27.2	29.6	26.2	24.7
BBzP	7.8	8.2	8.9	7.7	7.1	6.8	6.8	6.9
DEHP	285	300	299	298	298	273	277	298

DEP, diethyl phthalate; DnBP, di(n-butyl) phthalate; DiBP, di(isobutyl) phthalate; BBzP, butyl benzyl phthalate; DEHP, di(2-ethylhexyl) phthalate.

* $P < 0.05$ using Mann–Whitney and logistic regression (with phthalate divided into thirds). OR = 0.70.** $P < 0.05$ using logistic regression (with phthalate divided into thirds) and linear regression.^acurrent wheeze defined as more than 3 times within the last 12 months.^bWeighted phthalate concentration = $[(3.6 \times \text{phthalate concentration in home} + \text{phthalate concentration in day care})/4.6]$.

phthalate mass fractions in dust collected from a single environment (either the child's home or day care center) and using a weighted dust mass fraction that accounted for time spent both at home and at the day care facility. Mass fractions of DEP in the home and the weighted mass fractions in the two environments were higher for all DS case groups than for healthy controls. Median DnBP mass fraction in the home was lower in the asthma case group than in the healthy control group ($P < 0.05$); it was also lower in other groups (except for doctor-diagnosed allergic rhinoconjuncti-

tis), although without statistical significance. This tendency weakened when we accounted for both the home and day care center environments. No significant differences were found between mass fractions of DiBP and BBzP measured among any of the DS cases and healthy controls. For DEHP, we found higher mass fractions in the homes of DS case children compared with the healthy controls, but the difference was statistically significant only for the children with 'current wheeze' as reported by the parents. This significance was not retained for the association between

parental-reported current wheeze and the weighted DEHP mass fraction for homes and day care centers.

Discussion

The number of studies that investigate potential associations between measurement-based exposure assessments and clinically diagnosed allergic diseases is limited. Earlier studies often used questionnaires and surrogates for exposure to assess the relationship between children's health and indoor environmental factors. Such data may not be as reliable as measurement-based exposure assessments coupled with clinical health examinations (Hagerhed-Engman et al., 2007; Naydenov et al., 2008; Strachan, 1988). Studies on the impact of phthalates on allergic diseases in children are especially limited (Heinrich, 2011). The present study attempts to fill some of these gaps. It investigates associations between clinically confirmed allergic diseases and multiple, objectively assessed, indoor environmental factors (allergens, phthalates, nicotine, PAHs, and air change rates). The design of the study allowed a comparison between randomly selected healthy children (healthy controls) and children with various allergic diseases (disease-specific cases), the occurrence of which were confirmed using well-defined diagnostic criteria, clinical assessment, and determination of specific IgE sensitization. It is important to note that due to the cross-sectional nature of the study, significant associations between the measured environmental factors and allergic diseases may not reflect causal relationships.

Significantly higher air change rates were measured among the specific IgE-negative asthma cases compared with the specific IgE-positive asthma cases. The concentrations of dust mite allergens were higher in the homes of the specific IgE-positive group than in the homes of the specific IgE-negative group. These results may indicate the potential significance of ventilation in relation to sensitization among asthmatics. They further support earlier studies showing that increased ventilation may lead to decreased concentrations of certain indoor allergens and perhaps to clinical improvement in house dust mite-sensitive patients with asthma (De Blay et al., 1991; Eggleston, 2005; Eick and Richardson, 2011; Hagerhed-Engman et al., 2009; Harving and Korsgaard, 1994; Harving et al., 1993, 1994; Institute of Medicine, U.S., 2004; Takaro et al., 2011; Wickman et al., 1999). In contrast, Wright et al. (2009) found no significant difference in the reduction in house dust mite levels in homes with newly installed mechanical ventilation systems providing an additional 0.5 air changes per hour, compared with placebo-controlled homes.

Homes of children with asthma or allergic rhinoconjunctivitis had lower concentrations of cat allergens than homes of healthy controls. We found that the

median cat allergen concentration was significantly lower for the specific IgE-positive children with asthma compared with the specific IgE-negative children and with the healthy controls. Compared with the healthy control group, a smaller fraction of families with children with asthma indicated having a cat and a larger fraction indicated avoiding having a pet. Similar differences in pet-keeping habits were found among families of children with allergic rhinoconjunctivitis. Contrary to cat allergen, median dust mite allergen concentration was higher for the specific IgE-positive children with asthma compared with the specific IgE-negative children and with the healthy controls. These comparisons suggest deliberate cat avoidance by families of allergic children.

Polycyclic aromatic hydrocarbons were used in this study as indicators of traffic-related pollutants, which have been linked to asthma in earlier studies (Jerrett et al., 2008; McConnell et al., 2006; McCreanor et al., 2007; Salam et al., 2008; Zmirou et al., 2004). No associations were found between concentrations of PAHs in the dust collected from the homes and any DS case status based on the CEI, perhaps reflecting the relatively low PAH levels in the dust. Significant differences were also not found between specific IgE-negative and specific IgE-positive DS case children. Concentrations of nicotine measured in the settled dust were significantly higher among asthma cases compared with healthy controls. These results are in line with previous studies (Cook and Strachan, 1997; Gold, 2000; Pietinalho et al., 2009). Nicotine levels in the dust were not associated with any other health indicator in this study. This may be partly due to the relatively low nicotine levels in the investigated homes compared with levels reported in earlier studies (Weschler et al., 2011).

Several earlier studies have examined potential associations between phthalates in dust and asthma, rhinoconjunctivitis, and atopic dermatitis (Bornehag et al., 2004; Hsu et al., 2012; Kolarik et al., 2008). These studies indicated a positive relationship between BBzP and rhinitis and eczema, between DEHP and asthma, and between DEHP and wheezing in the last 12 months as reported by the parents. Our analyses did not confirm any significant positive associations between phthalate mass fractions and clinically diagnosed allergic diseases. Taking into account only mass fractions in the dust collected from the homes, median DEHP and DEP mass fractions were higher for the clinically diagnosed case groups than for the healthy controls. The differences were, however, not significant. Additionally, we found a significant negative association between asthma case status and DnBP. Median weighted dust mass fractions of DEP and DEHP from both the homes and day care centers tended to be higher for the three clinically diagnosed case groups than for the healthy control group.

Median weighted dust mass fractions of DnBP and DiBP were higher among the atopic dermatitis cases than among the healthy controls. None of the differences were significant. Analyzing for health indicators reported by the parents in the written questionnaire, we found a positive association between DEHP in the house dust and wheeze. Such association has been found in a previous study by Kolarik et al. (2008). The association did not remain significant after accounting for DEHP in the dust collected both from the homes and day care centers. These results indicate the potential importance of more completely accounting for children's exposures occurring in various environments when investigating health effects of indoor environmental factors. Further analyses on the association between urinary phthalate metabolites, as indicators of total phthalate exposures, and allergic diseases in these children will be reported in the near future.

The questionnaire data (Table S1) revealed that among children with current wheeze, the concentrations of dog allergens were higher ($P < 0.05$) compared with the healthy controls. Among children with doctor-diagnosed allergic rhinoconjunctivitis reported by the parents, the concentrations of two PAHs (BAA and BAP) were lower compared with healthy controls and Der f1 dust mite allergens, nicotine, and PAHs were lower for the specific IgE+ cases compared with healthy controls. The discrepancies between results based on the questionnaire data and those based on the CEI data underline the importance of using reliable disease assessment in future studies.

Conclusion

In this study, we looked for associations between indoor environmental factors and clinically confirmed allergic diseases in children. We found that the air change rates in the randomly selected healthy control group were lower than in the homes of children with a clinical diagnosis of asthma, allergic rhinoconjunctivitis, or atopic dermatitis. However, among children with asthma, significantly lower air change rates and higher levels of house dust mite allergens were mea-

sured for those who were IgE-sensitized. These findings are consistent with low ventilation rates leading to increased levels of certain allergens in the indoor environment, which may trigger sensitization and development of allergic diseases. On the other hand, lower concentrations of cat and dog allergens were measured among children with asthma compared with healthy controls. IgE-sensitized asthma cases had lower concentrations of cat allergens in their homes than non-sensitized asthma cases. Additional information obtained from the questionnaire indicated active cat avoidance behavior from birth or debut of symptoms. Concentrations of nicotine in the dust were significantly higher among asthma cases than among healthy controls. Phthalate mass fractions in the dust collected from the children's homes and day care centers were not associated with any disease-specific case status. However, we found a significant association between DEHP in dust and parent-reported current wheeze.

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Supporting Information

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

Table S1. Dust allergen concentrations, air change rates, bedroom volume and concentrations of nicotine, and polycyclic aromatic hydrocarbons in the dust (median values) in the homes of 242 randomly selected children who were clinically confirmed to be without allergic disease (healthy controls), compared with children with doctor-diagnosed asthma, allergic rhinoconjunctivitis, atopic dermatitis, or current wheeze as reported by the parents in the written questionnaire.

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Supporting Information

Associations between selected allergens, phthalates, nicotine, PAHs and bedroom ventilation and clinically confirmed asthma, rhinoconjunctivitis and atopic dermatitis in preschool children

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Table S1. Dust allergen concentrations, air change rates, bedroom volume and concentrations of nicotine and polycyclic aromatic hydrocarbons in the dust (median values) in the homes of 242 randomly selected children who were clinically confirmed to be without allergic disease (healthy controls), compared to children with doctor diagnosed asthma, allergic rhinoconjunctivitis, atopic dermatitis or current wheeze as reported by the parents in the written questionnaire.

	N	Der p1 (ng/g)	Der f1 (ng/g)	Der p2 (ng/g)	Fel d1 (ng/g)	Can f1 (ng/g)	ACH	Bedroom vol (m ³)	Nicotine (µg/g)	Pyrene (µg/g)	BAA (µg/g)	BAP (µg/g)
Healthy controls	242	110.0	977.2	121.5	114.5	81.6	0.39	25.1	0.87	0.12	0.017	0.009
Asthma	110	116.1	769.1	111.6	128.0	71.0	0.44	22.8	1.28	0.12	0.018	0.008
IgE negative	53	108.3	471.8	105.3	166.8	91.2	0.44	21.9	1.18	0.12	0.017	0.008
IgE positive	29	127.2	1914	165.6	125.1	54.3	0.40	23.5	1.29	0.11	0.018	0.010
Current wheeze	68	133.1	782.0	123.9	141.6	106.8*	0.42	22.4	1.60	0.12	0.016	0.007
Allergic rhinoconjunctivitis	56	103.7	881.1	150.5	83.1	48.9	0.56	23.5	0.09	0.13	0.014*	0.007*
IgE negative	13	651.0	470.7	191.4	120.5	61.9	0.30	20.8	-	0.13	0.012	0.006
IgE positive	30	49.6	582.7*	98.2	99.6	61.5	0.47	25.8	0.09*	0.12	0.016*	0.008*
Atopic dermatitis	83	137.7	637.1	109.4	158.5	52.3	0.47	23.6	0.62	0.14	0.018	0.008
IgE negative	35	143.6	410.6	80.1	110.7	54.4	0.44	21.7	0.82	0.15	0.017	0.010
IgE positive	27	107.9	785.5	101.6	182.4	52.3	0.47	24.7	0.09	0.16	0.018	0.009

Der p1, Der f1, Der p2: dust mite allergens; Fel d1: cat allergen; Can f1: dog allergen; ACH: air change rates per hour; BAA: Benz(a)-anthracene; BAP: Benzo(a)-pyrene; IgE positive: when any of the 23 measured serum specific Immunoglobulin E were above 0.35 kU/L.

* p<0.05, using Mann Whitney U test comparing to the healthy control group