

ORIGINAL PAPER

Early skin sensitization to aeroallergens

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Summary

Background Early detection of aeroallergen sensitization is important as a prognosis factor but may be more difficult in young children.**Objective** We sought to demonstrate that skin sensitization to aeroallergens was present in a selected group of 0–2-year-old children and that it was associated with environmental exposure and a family history of allergic disease.**Methods** Data on exposure and history were extracted from the files of 824 children seen in the asthma clinic and who were skin tested to a panel of aero- and food allergens.**Results** Forty percent of our children demonstrated atopy, 28% were sensitized to aeroallergens, the majority of which to house dust mite. Higher sensitization rates were found in children with large weals to histamine ($P < 0.001$) and in those who slept with soft toys [odds ratio (OR) 1.45, 95% confidence interval (CI) 1.02–2.08]. With a definition of sensitization including the size of the weal to histamine, there was a negative association with a personal history of eczema only (OR 0.66, 95% CI 0.45–0.99). There was no gender-dependent effect and no association with day-care attendance.**Conclusion** This is one of the largest studies to evaluate skin testing in a selected population of young children. We found a high prevalence of sensitization to aeroallergens, which was associated with exposure to soft toys. Further follow-up of this population will allow assessment of the predictive value of this sensitization.**Keywords** aeroallergens, allergy, infants, skin prick tests, young children*Submitted 10 September 2007; revised 22 November 2007; accepted 2 December 2007*

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Introduction

Allergic disorders such as asthma and eczema are very common in childhood and their prevalence is still increasing, especially in younger children [1]. Their clinical evolution may follow a pattern termed the 'allergic march', which describes the development of food allergy and eczema in early life, later replaced by aeroallergen sensitization associated with asthma and rhinitis [2, 3].

As many as 60% of children wheeze at least once before the age of 3 years, but only a small proportion of these children will develop an asthmatic phenotype [4, 5]. Furthermore, at least half of the adults with asthma developed their first respiratory episode before the age of 3, and 80% had symptoms before 6 years of age [5, 6]. Thus, early diagnosis is important if we are to design and implement prevention strategies. Longitudinal cohort studies such as the Tucson study [7] have defined different

phenotypes in children with preschool wheezing and demonstrated that one of the major factors associated with the persistence of respiratory symptoms in later childhood and adulthood is atopy and particularly sensitization to aeroallergens [8, 9]. Recently published data from the German MAS study confirmed the importance of early sensitization to aeroallergens for the development of chronic asthma [10].

Skin prick testing (SPT) is often considered to be the standard method to determine allergic sensitization, and guidelines have been edited to improve standardization world-wide [11, 12]. However, the diagnosis of atopy is often difficult in infants and young children because skin reactivity may be reduced, particularly below 6 months of age [13]. Furthermore, sensitization to aeroallergens has often been reported to appear after 2 years of age [2, 11] and transient sensitization has been described in 20% of these young children [14, 15]. Thus, the

majority of cohort studies report results from skin tests performed after 2–3 years of age or from specific IgE mainly to food allergens.

Environmental factors and in particular exposure to indoor allergens interact directly or indirectly with the genetic background of any individual to promote the development of allergic sensitization and atopic disease such as asthma [16].

Thus, there is a lack of large studies evaluating skin sensitization to aeroallergens in infants related to exposure and a family history of allergic disease.

We report the results of skin testing, especially with regard to sensitization to aeroallergens, in 824 infants and young children below 2 years of age, followed in a respiratory clinic of a tertiary centre in Belgium. Associations with environmental exposure and a family history of allergic disease are described.

Methods

Patients

All children aged below 2 years seen in the asthma clinic between 15 January 1998 and 15 July 2002 and who underwent SPT for clinical reasons were included in this study. The following data were extracted from the files: age, gender, cat/dog ownership, soft toys in the bed, wall-to-wall carpet in the bedroom, exposure to environmental tobacco smoke, a familial (mother, father or sibling) history of atopy (asthma, eczema or hayfever), a personal history of eczema, day care attendance, number of siblings and indication for skin testing. A child was said to have a positive history of eczema if he/she had been diagnosed by a doctor or if the clinical examination when seen at the clinic was compatible with this diagnosis.

Skin testing

SPTs were performed by one of three nurses working in the clinic using the method described by the EAACI [11, 17]. Standardized solutions from Stallergens (Alyostal, Stallergens, Belgium, SA) were used for all allergens, except for milk, where fresh semi-skimmed milk was used. Histamine (10 mg/mL histamine dihydrochloride) was used as the positive control and 50% glycerol saline solution as the negative control. Solutions were placed on the skin of the child's back and pricked with single-use lancets and the tests were read after 20 min. The weal size was recorded as the mean of the largest diameter and the diameter at 90° to this. A test was considered to be positive if the mean weal diameter was at least 3 mm larger than the negative control. The following allergens were tested: *Dermatophagoïdes pteronyssinus*, *Dermatophagoïdes farinae*, cat, dog, feathers, grass pollen, milk, egg, cod

and peanut. For the purposes of this study, a child was declared to be atopic if he had a positive reaction on skin testing for any of these aero- and food allergens. Further analyses were performed with a second definition of a positive skin test, for which the weal had to be >3 mm diameter and at least half the size of the positive control, as proposed by some authors [14].

Data analysis

All data were double checked and entered on a spreadsheet. Statistical analyses were performed using SPSS statistical software (SPSS 15.0, Chicago, IL, USA). Numerical variables (age, size of the weal to the positive control) were expressed as mean $\pm$ SD and by medians and interquartile ranges, and were compared by the Wilcoxon's rank sum test. Categorical variables (gender, soft toys in the child's bed, day care, exposure to environmental tobacco smoke, a family history of atopy, the presence of a dog/cat in parental house, a personal history of eczema, family size and carpet in child's bedroom) were analysed by a χ^2 test, and their association with skin sensitization to aeroallergens was quantified by relative risk with a 95% confidence interval (CI) estimated by the Miettinen method. The independent influence of these various parameters was studied by multivariate logistic regression with backward selection of variables by Wald and likelihood ratio tests ($P < 0.10$), and was quantified by odds ratio (OR) and 95% CIs. All statistical tests were two tailed.

The associations between skin sensitization to aeroallergens and day-care attendance were analysed separately for boys and girls to assess the gender effect separately as described in the PIPO study [18].

Results

Demographic data (Tables 1 and 2)

We assessed 889 children. Of these, 65 were excluded for the following reasons: no skin tests against aeroallergens ($n = 34$), results of the skin tests not found in the files ($n = 22$) and no data on environmental exposure in the files ($n = 9$). The indication for skin testing was mainly recurrent respiratory tract infections with or without wheeze and/or cough (84.3%). Based on our data, it was not always possible to determine precisely whether the child had wheezing episodes with these respiratory infections. The remaining chief complaints were eczema (9.5%), gastrointestinal symptoms (5.2%) and urticaria (1.1%). All our children were below 2 years of age with a median age of 1 year. As usual in this setting, we had a large majority of boys (63.2%). Wall-to-wall carpeting was rarely found (14.8%) but young children often slept with soft toys (69.2%). Exposure to tobacco smoke

Table 1. Demographical data and correlation with skin sensitization to aeroallergens for age and size of the weal to positive control

<i>n</i> = 824	Mean (± SD)	Median (IQR)	Mean (± SD) if not sensitized	Mean (± SD) if sensitized	<i>P</i> -value
Age (year)	1.05 (± 0.45)	1.00 (0.67; 1.42)	1.04 (± 0.45)	1.08 (± 0.43)	0.15
Positive control (weal diameter in millimetres)	6.8 (± 4.4)	6 (5; 8)	6.5 (± 4.5)	7.7 (± 3.9)	<0.001

IQR, interquartile range.

Table 2. Demographical data and correlation with skin sensitisation to aeroallergens for categorical variables

	% Positive	Prevalence positive/ negative	% Sensitized if criteria (–)	% Sensitized if criteria (+)	<i>P</i> -value	RR (95% CI)
Soft toys in child's bed	69.2	529/235	23.0	30.2	0.04	1.30 (1.01–1.66)
Gender = male	63.2	523/301	28.9	27.2	0.59	0.94 (0.75–1.18)
Day-care attendance	59.5	360/245	26.9	28.6	0.66	1.08 (0.76–1.56)
Exposure to ETS	46.8	356/404	27.0	30.1	0.35	1.16 (0.85–1.59)
Family history of atopy	38.6	313/497	28.1	27.8	0.91	0.98 (0.72–1.34)
Dog present in parental house	34.3	277/530	26.0	31.0	0.13	1.28 (0.93–1.76)
Family size						
0 vs. 1 sibling	33.8	251/174/317	25.9	26.5	0.63	1.03 (0.71–1.51)
0 vs. ≥2 siblings				29.9		1.22 (0.79–1.88)
Personal history of eczema	29.6	242/575	27.8	28.1	0.94	1.01 (0.72–1.43)
Cat present in parental house	24.9	201/606	27.7	27.9	0.97	1.01 (0.66–1.54)
Carpets in the child's bedroom	14.8	119/683	27.1	31.1	0.37	1.22 (1.80–1.85)

ETS, environmental tobacco smoke; CI, confidence interval.

was present in almost half of these families. Day-care attendance was also frequent (59.5%). A personal history of eczema was found in 29.6% of the children and, as stated above, was the chief complaint for 9.5% of them.

Sensitization (Table 3)

Only 18 children (2.2%) had a weal of <3 mm to positive control (hypo-reactivity). Sixty nine of our patients (8.4%) had a weal for the negative control ≥2 mm (hyper-reactivity or dermographism), but 19 of them reacted significantly to at least one allergen (weal 3 mm above negative control). Thus, skin testing was inconclusive in 68 children (8.3%).

Almost 40% (326) of our patients were sensitized to at least one aero or food allergen. Of these, 70.9% (*n* = 231) were sensitized to at least one aeroallergen. The allergen to which our patients were most often sensitized was egg (43.3% of the children with atopy), followed by *D. pteronyssinus* (27.9%) and grass pollen (17.8%). Between 13% and 15% of the children demonstrating atopy were sensitized to each of the remaining aeroallergens. Few children were sensitized to milk and other food allergens (between 9.5% and 11.0% of the children with atopy), which may be due to the population studied, mainly with respiratory symptoms.

Table 3. Skin prick tests results (*n* = 824)

Allergen	<i>N</i> (percentage of children tested)	Positive, in percentage of children demonstrating atopy
Any allergen	326 (39.6)	
Any aeroallergen	231 (28.0)	70.9
Egg	141 (17.1)	43.3
<i>Dermatophagoides pteronyssinus</i>	91 (11.0)	27.9
Grass pollen	58 (7.0)	17.8
Feathers	48 (5.8)	14.7
Dog	48 (5.8)	14.7
Cat	45 (5.5)	13.8
<i>Dermatophagoides farinae</i>	44 (5.3)	13.5
Milk	36 (4.4)	11.0
Fish (cod)	36 (4.4)	11.0
Peanut	31 (3.8)	9.5

Children sensitized to aero and food allergens in absolute numbers (percentage of all children tested) and percentage of children demonstrating atopy.

Associations with exposure

The risk of a child being sensitized to at least one aero-allergen was higher if the size of their response to the positive control was large (*P* < 0.001, mean weal size for positive control if sensitized to aeroallergen: 7.7, if not:

6.5) (Table 1). Seventy four percent of children with a response to the positive control above 10 mm were sensitized to at least one aero-allergen. Children were also more likely to be sensitized to aeroallergens if they slept with a soft toy (30.2% vs. 23.0%, $P=0.04$) (Table 2) and this was still significant by multivariate analysis (OR 1.45, 95% CI: 1.02–2.08). The association with age was not significant ($P=0.15$) but there was a trend for children below 6 months to be less often atopic than older children (30.8% vs. 44.0%, $P=0.06$).

Further analyses were performed with a second definition of what constitutes a positive skin test. With this definition, 20.0% ($n=165$) of the population (vs. 27.8%) was sensitive to at least one aeroallergen. Not unexpectedly, the association with the size of the positive control disappeared. The association between sensitization to aeroallergen and the presence of soft toys in the bed was not significant (17.0% of children without soft toys were sensitized to aeroallergens vs. 21.6% of those who had soft toys) but the personal history of eczema seemed to play a protective role against skin sensitization to aeroallergens and this association was still significant in the multivariate model (OR 0.66, 95% CI 0.45–0.99).

None of these associations was found to be significant when boys and girls were analysed separately.

Discussion

To our knowledge, this is one of the largest studies to evaluate skin sensitization to aeroallergens in infants aged below 2 years. We have demonstrated that, in our selected population, there was a high prevalence of sensitization to aeroallergens, mainly to indoor perennial allergens such as house dust mite (HDM) but also seasonal allergens such as grass pollen. Sleeping with soft toys was associated with a greater risk for sensitization to aeroallergens and children sensitized to aeroallergens had larger histamine weals.

One strength of this study is that all skin tests were performed by the three nurses, trained for skin testing, attached to our clinic, thereby probably reducing inter-rater variability. The mean weal diameter for histamine was similar to the reported guidelines [19]. We did not evaluate the intrarater variability by duplicate tests as it was not deemed feasible in the clinical setting of the study and is usually not advised in infants.

We believe that this heterogeneous population of children with chronic or recurrent cough associated or not with wheezing reflects the children usually seen in the setting of a respiratory clinic.

Data on exposure to allergens were collected contemporaneously but we only evaluated this exposure to aeroallergens based on questionnaire data and this surrogate measure may introduce a bias as no measurement of actual exposure in the indoor air was made.

Despite the young age of our patients, the majority of skin tests could be interpreted. We found hypo- or hyper-reactivity of the skin in fewer than 10% of our infants.

The high prevalence of atopy as defined in this study (40%) and sensitization to aeroallergens (28%) is due to the population selected. Population-based studies such as that performed by Vasar et al. [20] in Estonia and Sweden find a lower prevalence of atopy (7% at 6, 12 and 24 months). Dean et al., in a nested cohort followed up from birth and tested at 1, 2 and 3 years, found sensitization to food allergens, respectively, in 2.8%, 3.9% and 3.7% of the children. At the same ages, the prevalence of sensitization for aeroallergens was, respectively, 1.3%, 6.4% and 10.7% [21, 22]. In a review on skin testing and sensitization to inhalant allergens in Europe for the GA2LEN project, Heinzerling et al. [23] found sensitization rates of 4–20% to *D. pteronyssinus* in population-based studies (compared with 11% in our study) and sensitization to *D. pteronyssinus* varied from 10% of positive skin tests in Norway to 67% in Australia and New Zealand (compared with 27.9% in our study).

Selecting a high-risk cohort of 2–3-year-old-children with wheeze in the PEAK study, Guilbert et al. [8] performed SPTs and found a high prevalence of sensitization to aeroallergens (54%). Arshad et al. [24] evaluated the skin test reactivity in their birth cohort at the age of 4 years and found sensitization to aeroallergens in 9.2% of their subjects (prevalence of atopy 9.4%). In the subgroup of children with asthma, the prevalence of atopy was 44%. It is noticeable that the majority of these children were still atopic when tested at 10 years of age [25]. In a cohort of children born to atopic parents, Lemasters et al. [26] described a prevalence of atopy of 28% at 1 year and the prevalence of aeroallergen sensitization was 18%.

In these studies as in ours, a positive response was defined as a weal of ≥ 3 mm weal, as recommended by the EAACI subcommittee on allergen standardization and skin tests [11] and as is current practice in most European countries [23].

Thus, cohort studies with similar inclusion criteria have found the prevalence of atopy and sensitization to aeroallergens to be similar to our data although they often evaluated children at an older age.

It is important to detect sensitization to aeroallergens early because large longitudinal cohort studies have confirmed that it is the most important risk factor for the persistence of respiratory symptoms [5, 7, 8, 27]. The latest results from the German MAS study also confirm these data [10]. Although they used specific IgE as a marker of sensitization, they showed that lung function at ages 7, 10 and 13 years was significantly reduced in children with early wheezing when sensitized to perennial aeroallergens early in life, especially if they were exposed to high levels of the respective allergens. Similarly, children sensitized

to perennial allergens were prone to developing a chronic course of asthma characterized by more severe symptoms.

We have shown that sensitization to aeroallergens was associated with environmental exposure as it was more prevalent in infants who slept with soft toys, a proxy for high levels of HDM. This is in accordance with studies showing that exposure to high levels of HDMs increases the risk for sensitization [28].

Children sensitized to aeroallergens had larger histamine weals in our study. This has already been described by other authors [19] and these authors have concluded that atopic children had larger histamine weals rather than children with large weals being more likely to be diagnosed as having atopy. Thus, they demonstrated that when the degree of allergy was reduced, as by immunotherapy, the histamine reactivity of the skin was reduced. When using another definition of aeroallergen sensitization based on the size of the weal to positive control [14] we did not find any association with exposure to allergens but a negative association with a positive history of atopic dermatitis in keeping with the diagnosis of sensitization only based on the size of the weal. Thus, we can confirm that atopic children develop larger weals to histamine.

There was no apparent protective role of day-care attendance or large family size in the development of sensitization to aeroallergens in our group. The so-called hygiene hypothesis emerged from data showing that children with older siblings were less prone to developing allergic sensitization [29], further confirmed by data showing that early attendance to day-care centres was associated with less atopic symptoms [30, 31]. Recently, some studies have challenged this association [32] and sometimes an inverse relationship has been demonstrated [33].

In the PIPO study in Belgium, Hagendorens et al. [18] measured specific IgE levels in 810 infants at 1 year of age and found that day care attendance was negatively associated with early sensitization only in boys. We were not able to confirm a gender-dependent effect of environmental factors or a familial history of atopy in our subjects.

In conclusion, in a study of 824 children attending an asthma clinic we have confirmed that aeroallergen sensitization was common at this early age, and was associated with high exposure to soft toys and with the size of the weal to positive control. We were not able to confirm any association with day care attendance nor any gender effect. Further follow-up of these children will allow us to assess the predictive value of early sensitization to aeroallergens.

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References

- 1 The International Study of Asthma and Allergy in Childhood (ISAAC) Steering Committee. Worldwide variation in prevalence of symptoms of asthma, allergic rhinoconjunctivitis, and atopic eczema: ISAAC. *Lancet* 1998; **351**:1225–32.
- 2 Kulig M, Bergmann R, Klettke U, Wahn V, Tacke U, Wahn U. Natural course of sensitization to food and inhalant allergens during the first 6 years of life. *J Allergy Clin Immunol* 1999; **103**:1173–9.
- 3 Sigurs N, Hattevig G, Kjellman B, Kjellman NI, Nilsson L, Björkstén B. Appearance of atopic disease in relation to serum IgE antibodies in children followed up from birth for 4 to 15 years. *J Allergy Clin Immunol* 1994; **94**:757–63.
- 4 Martinez FD, Wright AL, Taussig LM, Holberg CJ, Halonen M, Morgan WJ. Asthma and wheezing in the first six years of life. The Group Health Medical Associates. *N Engl J Med* 1995; **332**:133–8.
- 5 Phelan PD, Robertson CF, Olinsky A. The Melbourne asthma study: 1964–1999. *J Allergy Clin Immunol* 2002; **109**:189–94.
- 6 Sears MR, Greene JM, Willan AR et al. A longitudinal, population-based, cohort study of childhood asthma followed to adulthood. *N Engl J Med* 2003; **349**:1414–22.
- 7 Martinez FD. What have we learned from the Tucson children's respiratory study? *Paediatr Respir Rev* 2002; **3**:193–7.
- 8 Guilbert TW, Morgan WJ, Zeiger RS et al. Atopic characteristics of children with recurrent wheezing at high risk for the development of childhood asthma. *J Allergy Clin Immunol* 2004; **114**:1282–7.
- 9 Kotaniemi-Syrjänen A, Reijonen TM, Romppanen J, Korhonen K, Savolainen K, Korppi M. Allergen-specific immunoglobulin E antibodies in wheezing infants: the risk for asthma in later childhood. *Pediatrics* 2003; **111**:e255–61.
- 10 Illi S, von Mutius E, Lau S, Niggemann B, Gruber C, Wahn U. Perennial allergen sensitisation early in life and chronic asthma in children: a birth cohort study. *Lancet* 2006; **368**:763–70.
- 11 Dreborg S. The European Academy of Allergy and Clinical Immunology. Position paper: allergen standardization and skin tests. *Allergy* 1993; **48**:48–82.
- 12 Host A, Andrae S, Charkin S et al. Allergy testing in children: why, who, when and how? *Allergy* 2003; **58**:559–69.
- 13 Menardo JL, Bousquet J, Rodiere M, Astruc J, Michel FB. Skin test reactivity in infancy. *J Allergy Clin Immunol* 1985; **75**:646–51.
- 14 Karila C. [Allergen skin tests. Asthmatic infants and young children]. *Arch Pediatr* 2002; **9** (Suppl. 3):338s–43s.
- 15 Julge K, Vasar M, Björkstén B. Development of allergy and IgE antibodies during the first five years of life in Estonian children. *Clin Exp Allergy* 2001; **31**:1854–61.
- 16 Martinez FD. Genes, environments, development and asthma: a reappraisal. *Eur Respir J* 2007; **29**:179–84.
- 17 Dreborg S. The European Academy of Allergology and Clinical Immunology. Skin tests used in type I allergy testing. Position paper. *Allergy* 1989; **44** (Suppl. 10):1–59.

- 18 Hagendorens MM, Bridts CH, Lauwers K *et al*. Perinatal risk factors for sensitization, atopic dermatitis and wheezing during the first year of life (PIPO study). *Clin Exp Allergy* 2005; **35**: 733–40.
- 19 Dreborg S. Histamine reactivity of the skin. *Allergy* 2001; **56**: 359–64.
- 20 Vasar M, Julge K, Bjorkstein B. Development of atopic sensitization and allergic diseases in early childhood. *Acta Paediatr* 2000; **89**:523–7.
- 21 Dean T, Venter C, Pereira B *et al*. Patterns of sensitization to food and aeroallergens in the first 3 years of life. *J Allergy Clin Immunol* 2007; **120**:1166–71.
- 22 Venter C, Pereira B, Grundy J *et al*. Incidence of parentally reported and clinically diagnosed food hypersensitivity in the first year of life. *J Allergy Clin Immunol* 2006; **117**:1118–24.
- 23 Heinzerling L, Frew AJ, Bindslev-Jensen C *et al*. Standard skin prick testing and sensitization to inhalant allergens across Europe – a survey from the GALEN network. *Allergy* 2005; **60**:1287–300.
- 24 Arshad S, Tariq S, Matthews S, Hakim E. Sensitization to common allergens and its association with allergic disorders at age 4 years: a whole population birth cohort study. *Pediatrics* 2001; **108**:e33.
- 25 Kurukulaarachy RJ, Matthews S, Arshad SH. Defining childhood atopic phenotypes to investigate the association of atopic sensitization with allergic disease. *Allergy* 2005; **60**:1280–6.
- 26 LeMasters G, Wilson K, Levin L *et al*. High prevalence of aeroallergen sensitization among infants of atopic parents. *J Pediatr* 2006; **149**:505–11.
- 27 Kotaniemi-Syrjanen A, Reijonen TM, Korhonen K, Korppi M. Wheezing requiring hospitalization in early childhood: predictive factors for asthma in a six-year follow-up. *Pediatr Allergy Immunol* 2002; **13**:418–25.
- 28 Platts-Mills TA, Vervloet D, Thomas WR, Aalberse RC, Chapman MD. Indoor allergens and asthma: report of the Third International Workshop. *J Allergy Clin Immunol* 1997; **100**:S2–24.
- 29 Strachan DP. Hay fever, hygiene, and household size. *BMJ* 1989; **299**:1259–60.
- 30 von Mutius E, Martinez FD, Fritzsche C, Nicolai T, Reitmeir P, Thiemann HH. Skin test reactivity and number of siblings. *BMJ* 1994; **308**:692–5.
- 31 Ball TM, Castro-Rodriguez JA, Griffith KA, Holberg CJ, Martinez FD, Wright AL. Siblings, day-care attendance, and the risk of asthma and wheezing during childhood. *N Engl J Med* 2000; **343**:538–43.
- 32 Balemans WA, Rovers MM, Schilder AG *et al*. Recurrent childhood upper respiratory tract infections do not reduce the risk of adult atopic disease. *Clin Exp Allergy* 2006; **36**: 198–203.
- 33 Hagerhed-Engman L, Bornehag CG, Sundell J, Aberg N. Day-care attendance and increased risk for respiratory and allergic symptoms in preschool age. *Allergy* 2006; **61**:447–53.