

The most common phenotypes of sensitization to inhalant allergens in childhood

K. STORM VAN'S GRAVESANDE, M. MOSELER and J. KUEHR

University Children's Hospital, Freiburg, Germany

Summary

Background Atopic individuals are frequently sensitized to a limited number of certain allergens, although most of them are exposed to multiple inhalant allergens in daily life.

Objective We investigated the hypothesis that observed common patterns of sensitization might occur with similar frequency within two independent study populations of school-children.

Methods The results were derived from skin-prick tests conducted on two large samples of children (study 1: $n = 583$; study 2: $n = 1099$) examined with the same panel of six inhalant allergens.

Results In order to ensure that the comparison was uniform, the younger subpopulation of study 1 ($n = 147$) was compared with the sample of study 2 ($n = 374$). The highest frequency for monosensitization was found for sensitization to *Dermatophagoides pteronyssinus* (study 1: 18.4%, study 2: 20.3%), followed by monosensitization to grass pollens (study 1: 12.2%; study 2: 8.8%). Using multiple logistic regression for each specific sensitization, a significantly increased relative risk of sensitization to hazel pollens (study 1 OR 5.9; study 2 OR: 24.3) appeared to be associated with sensitization to birch pollens. The same applied to dog dander (study 1 OR: 7.3; study 2 OR: 2.6), which showed an association with sensitization to cat dander.

Conclusion In summary, our data suggest that certain clusters of monosensitization and polysensitization to common inhalant allergens exist among a given population. This may well be a reflection of diversity in disposition to specific sensitization and/or antigen crossreactivity. From a practical point of view the data also might help in counselling parents of allergic children.

Keywords: allergens, allergy, children, combinations, crossreactivity, monosensitization, patterns, skin-prick test

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Introduction

It is generally accepted that atopic allergy has a multifactorial origin and frequently causes asthma and rhinitis in sensitized individuals. Predisposition to allergies is genetically determined and development of sensitization requires allergen exposure [1–5]. Therefore, genetic and environmental factors might be decisive for the specificity of IgE response. Individuals with clinical allergic disease are

frequently sensitized to a limited number of certain allergens, although most of them are exposed to multiple inhalant allergens in daily life. It is still not clear whether IgE immune responsiveness to a specific allergen is associated with sensitization to another allergen and, if so, how frequently such combinations occur among a given population.

Thus, the purpose of this study was to investigate patterns of sensitization to common inhalant allergens. Data from two large epidemiological studies were analysed. In an initial explorative stage using data from study 1, frequent patterns of sensitization which had been found in a sample

Correspondence Dr K. Storm van's Gravesande, University Children's Hospital, Mathildenstrasse 1, D-79106 Freiburg, Germany.

of schoolchildren were arbitrarily defined. An attempt was then made to confirm the findings using data from study 2.

Materials and methods

Population

The data were collected from two different epidemiological studies in southwestern Germany, both of which included skin-prick tests (SPT) on children of school age. For study 1 a population of 704 schoolchildren from the urban area of Freiburg and a community in the Black Forest was randomly selected in October 1987. A total of 587 children took part in all three SPTs performed at 1-year intervals [6]. The population of study 1 was restricted to only one child (oldest or youngest) from each family in order to ensure that children analysed were genetically independent.

In study 2, three SPTs were performed at yearly intervals on a population sample of initially 1470 schoolchildren from three towns in southwestern Germany who had started elementary school in 1989 [10].

SPT procedure

In both studies purified and immunochemically characterized aeroallergens (ALK, Denmark) were applied to the forearm (distance between allergens about 30 mm) in a standardized order with the aid of an uncoated Phazet[®] needle. Extracts of grass pollens (mixture of the six common species: *Avena elatior*, *Dactylis glomerata*, *Festuca pratensis*, *Lolium perenne*, *Phleum pratense* and *Poa pratensis*), birch pollens, hazel pollens, *Dermatophagoides pteronyssinus* (Dpt), cat and dog dander were used (concentration: 10 histamine equivalent in prick testing). Histamine hydrochloride (10 mg/mL) and sodium chloride (9 g/L) were applied as control solutions. The reaction was recorded after 15 min [7]. The largest and the perpendicular diameters of the weal reaction were measured and the arithmetic mean was calculated [8].

Classification of the SPT reaction

A definite sensitization to the allergen required a weal diameter of 2 mm and an allergen/histamine ratio ≥ 0.5 , or a weal reaction not fulfilling the above-mentioned criteria but occurring in at least the last two of three tests. In contrast, doubtful sensitization was defined as any weal reaction in at least one test not fulfilling the criteria for definite sensitization.

Statistical analysis

We tried to demonstrate that patterns of sensitization do not

occur randomly. Therefore, the relative frequencies of combinations were calculated for each sample. The association between sensitizations was described by relative risks using a specific sensitization as the dependent variable and each of the complementary sensitizations as independent variables. To estimate the relative risk of each complementary sensitization under mutual adjustment, multiple logistic regression was employed [9] and Odds Ratios (ORs) and 95% confidence intervals (95% CIs) were calculated.

Results

Study population

In study 1, 583 children aged 7–16 years (average of 11.3 years) took part in all three SPTs. A total of 374 children, 191 (51.1%) boys and 183 (48.9%) girls, with an average age of 10.6 years, was assigned to the group of 'younger children'; 362 children, 181 boys and 181 girls, were assigned to the group of 'older children' (average of 12.1 years). There was an overlap of 153 children between both subpopulations.

In study 2, 1099 children, 522 (47.5%) boys and 577 (52.5%) girls, aged 6–8 years (average age 7.6) underwent all three SPTs.

Comparison of sensitization in both study groups

The frequencies of doubtful and definite sensitization are displayed in Table 1. Comparing the results of both study populations, similar prevalences of definite and doubtful sensitization to different allergens were seen. The percentage of definite sensitization to Dpt was approximately equal in both samples (22.6% in study 1 and 21.9% in study 2). The percentage of definite sensitization to grass pollens in study 1 was about 10% higher than in study 2. However, the percentage of doubtful sensitization in study 2 was almost twice as high as in study 1. The group of 'older children' showed higher prevalences of sensitization than the group of 'younger children' regarding single allergens and regarding the aggregated results. ('at least one of the six allergens'). Taking higher prevalences in older children into account, the group of 'younger children' from study 1 and the total population of study 2 are compared in the following.

Percentage of monosensitization and polysensitization

The frequencies of numbers of different definite sensitizations in the subsamples of children with at least one sensitization (study 1, younger children $n = 147$; study 2 $n = 374$) were similar in both study samples (Table 2). Females were more frequently sensitized to one or two

Table 1. Percentage of reactivity to specific allergens

Sensitization to	Criterion	Study group/subgroup			
		Study 1			Study 2
		Total ¹ (n = 583)	Younger children ² (n = 374)	Older children ² (n = 362)	Total ¹ (n = 1099)
Dpt	Definite	22.6	21.1	24.0	21.9
	Doubtful	5.8	6.7	6.1	11.4
Grass pollens	Definite	27.8	27.3	29.6	18.7
	Doubtful	4.6	5.1	5.0	8.3
Birch pollens	Definite	19.9	19.5	20.2	14.0
	Doubtful	4.3	3.7	5.0	9.2
Hazel pollens	Definite	13.2	11.5	13.5	9.6
	Doubtful	5.8	5.1	6.9	11.1
Cat dander	Definite	17.7	17.6	19.1	15.7
	Doubtful	3.8	2.9	4.4	18.8
Dog dander	Definite	13.0	12.6	13.8	10.5
	Doubtful	9.3	8.0	10.5	18.6
At least one of the six allergens	Definite	40.3	39.3	43.9	34.0
	Doubtful	5.8	6.1	5.0	23.4

¹ all children with three SPTs;² overlap between both subpopulations (n = 153).

allergens, whereas males were more frequently sensitized to three, five, and six allergens.

Patterns of sensitization

With regard to 63 possible different combinations of sensitization, we identified the six most common patterns of sensitization, which represent about 50% of the cases (Table 3). Among the remaining half of the samples, other combinations of sensitization occurred each with a

single frequency up to 3.4%. Monosensitization to Dpt occurred with the highest prevalence in both study samples. A higher prevalence (12.2%) for monosensitization to grass pollens was found in study 1 compared with that in study 2 (8.8%). The frequencies of all other combinations were found to be similar in both study populations.

Monosensitization

The frequency of monosensitization expressed as a percentage

Table 2. Percentage for numbers of sensitization among study groups

Sum of different definite sensitizations	Study 1			Study 2		
	Total ¹ (n = 147)	Female (n = 59)	Male (n = 88)	Total (n = 374)	Female (n = 177)	Male (n = 197)
1	34.0	40.7	29.6	38.8	40.7	37.0
2	14.9	18.6	12.5	16.0	18.1	14.2
3	18.3	13.6	21.6	14.9	12.9	16.8
4	11.7	11.8	11.3	11.3	11.4	11.2
5	12.9	8.5	15.9	8.8	7.9	9.6
6	8.2	6.8	9.1	10.2	9.0	11.2

¹ the youngest child of each family.

Table 3. Combinations of sensitization (%)

Pattern of definite sensitization	Study 1			Study 2		
	Total (<i>n</i> = 147)	Female (<i>n</i> = 59)	Male (<i>n</i> = 88)	Total (<i>n</i> = 374)	Female (<i>n</i> = 177)	Male (<i>n</i> = 197)
Dpt only	18.4	18.6	18.2	20.3	19.8	20.8
Grass pollens only	12.2	16.9	9.1	8.8	10.2	7.6
Grass & birch pollens	6.1	6.8	5.7	3.5	5.1	2.0
Dpt & grass pollens & cat dander	4.8	5.1	4.5	2.4	1.1	3.5
Grass & birch & hazel pollens & dog and cat dander	5.4	3.4	6.8	1.3	1.8	1.0
All allergens	8.2	6.8	9.1	10.2	9.0	11.2
Other combinations of allergens	44.9	42.4	46.4	53.5	53.0	53.9

of all sensitizations to the same allergen of both study populations is displayed in Fig. 1. Monosensitization to Dpt is apparent with the highest frequency in both study samples (34%, *n* = 27 in study 1; 32%, *n* = 76 in study 2). Thus, about one-third of all individuals with sensitization to Dpt are monosensitized, whereas monosensitization to grass pollens and to birch pollens was less frequent.

Sensitization to more than one allergen

Combinations of sensitization were analysed employing multiple logistic regression. In order to analyse associations of different sensitizations only individuals with at least two sensitizations were included (Table 4). In both studies sensitization to grass pollens was associated with an additional sensitization to birch pollens (study 1 OR: 13.9 and study 2 OR: 3.4) and vice versa (study 1 OR: 13.77 and study 2 OR: 3.1). The same applied to the relationship between birch and hazel pollens and to the relationship between cat and dog dander. A negative association of co-sensitization to dog dander and birch pollens was found in both study populations.

In each model five tests for the different sensitizations are provided. Adjusting the *P*-level for the numbers of tests ($[\alpha = 0.05/5] = \alpha_{\text{adjusted}} = 0.01$), all results with ORs exceeding '1' and without inclusion of the value '1' in the 95% CI remained on a significant level.

Discussion

The objective of this analysis was to define patterns of sensitization to a panel of inhalant allergens using data from study 1 and to check consistency with data from study 2. Prevalences of sensitization and the frequencies of definite

sensitizations in study 1 were comparable to the findings in study 2, although they were on average higher in study 1. This might in part reflect the higher age of the sample in study 1. It has already been reported that prevalences of sensitization to inhalant allergens increase from early childhood to adulthood [10,11]. To take the increases of prevalences with age into account and to analyse genetically independent individuals, the sample of study 1 was restricted to the youngest child of each family.

To our knowledge only one previous investigation has addressed the coincidence of specific sensitization to common inhalant allergens [12]. In a Finnish population of 708 individuals aged 15–17 years, the participants were frequently sensitized to more than one allergen which belonged to clustering groups of allergens [13]. One cluster

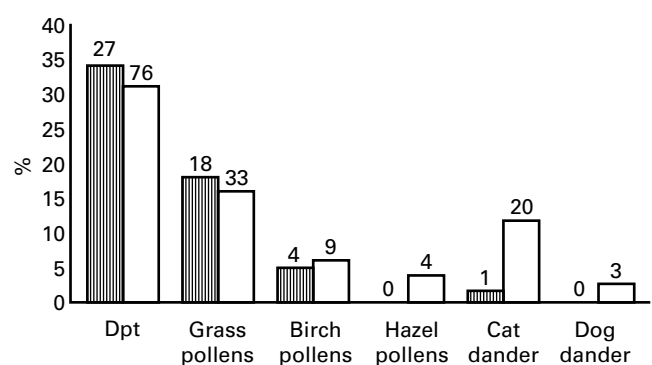


Fig. 1. Frequency of monosensitized children. This is shown as a percentage of those all sensitized to the same allergen. Numbers represent monosensitized children from both study samples. Study 1, the group of 'younger children' (*n* = 147), study 2 (*n* = 374). ▨, study 1; □, study 2.

Table 4. Odds ratio (OR and 95% CI) derived from logistic regression analysis. Predictors of sensitization in the population of study 1 ($n = 97$) and study 2 ($n = 229$) including children with at least two sensitizations

Predictor	Dependent variables									
	Sensitization to Dpt OR (95% CI)		Sensitization to grass pollens OR (95% CI)		Sensitization to birch pollens OR (95% CI)		Sensitization to hazel pollens OR (95% CI)		Sensitization to cat dander OR (95% CI)	
	Study 1 ($n = 45$)	Study 2 ($n = 64$)	Study 1 ($n = 13$)	Study 2 ($n = 57$)	Study 1 ($n = 28$)	Study 2 ($n = 84$)	Study 1 ($n = 54$)	Study 2 ($n = 127$)	Study 1 ($n = 32$)	Study 2 ($n = 76$)
Sensitization to Dpt	–	–	2.02 (0.45–9.04)	0.36 (0.14–0.90)	0.21 (0.06–0.71)	0.55 (0.23–1.30)	1.72 (0.69–4.29)	1.14 (0.54–2.41)	1.53 (0.54–4.37)	2.09 (1.07–4.10)
Sensitization to grass pollens	1.72 (0.44–6.74)	0.33 (0.13–0.83)	–	–	13.77 (2.47–76.89)	3.10 (1.35–7.11)	0.37 (0.09–1.48)	1.90 (0.79–4.55)	3.57 (0.76–16.80)	1.20 (0.57–2.51)
Sensitization to birch pollens	0.20 (0.06–0.67)	0.65 (0.29–1.46)	13.9 (2.59–75.22)	3.40 (0.84–5.03)	–	–	7.15 (2.09–24.50)	22.75 (9.20–56.26)	0.17 (0.04–0.69)	0.36 (0.17–0.77)
Sensitization to hazel pollens	1.79 (0.71–4.51)	1.22 (0.58–2.54)	0.41 (0.10–1.65)	2.05 (0.84–5.03)	5.87 (1.78–19.36)	24.34 (9.68–61.24)	–	–	1.69 (0.59–4.82)	2.01 (1.00–4.08)
Sensitization to cat dander	1.65 (0.58–4.70)	2.10 (1.07–4.12)	3.87 (0.84–17.79)	1.10 (0.52–2.32)	0.19 (0.05–0.77)	0.32 (0.15–0.69)	1.59 (0.56–4.51)	1.99 (0.95–4.17)	–	–
Sensitization to dog dander	0.95 (0.36–2.48)	2.13 (1.09–4.16)	1.01 (0.25–4.05)	0.91 (0.46–1.79)	2.31 (0.72–7.40)	0.80 (0.38–1.68)	1.29 (0.50–3.35)	1.83 (0.90–3.72)	7.27 (2.50–21.16)	2.59 (1.40–4.80)
Gender	0.53 (0.21–1.31)	0.43 (0.23–0.81)	0.88 (0.21–3.61)	1.34 (0.67–2.65)	0.79 (0.24–2.64)	1.01 (0.49–2.10)	1.05 (0.42–2.60)	0.97 (0.50–1.88)	1.46 (0.51–4.15)	1.09 (0.59–2.01)
									0.53 (0.21–1.36)	0.80 (0.45–1.40)

was formed by pollens and the second by dust allergens. Remarkably tight clustering in the above-mentioned study was found between allergens of some animal epithelium and house dust preparations. The current analysis revealed two predominant phenotypical characteristics at the population level. Firstly we found surprisingly high prevalences of monosensitization, and secondly we saw predominance of some associations of sensitizations. Both findings should be discussed in terms of genetic and environmental influences, since the interplay of both might determine sensitization to inhalant allergens [13, 14]. It has been generally accepted that allergen exposure is a prerequisite for the generation of allergy [15]. All children of both study populations had been living under identical geographical and climatic conditions for years. A uniform level of long-term exposure to common inhalant allergens might have been the consequence. In contrast, specific sensitization to environmental allergens tested by us showed different prevalences among the study samples. Thus, the presence of specific sensitization and frequencies of mono- and polysensitization may not primarily reflect allergen exposure. Although allergen exposure is a risk factor for sensitization, the exposure history might not be a predictor for sensitization.

It is more likely that our findings regarding monosensitization and clustering of different sensitizations could be explained by two other approaches. Firstly, the ability of the immune system to recognize antigens requires an appropriate counterpart within the T-cell receptor repertoire. Production of specific IgE antibodies may be constrained by variation in the T-cell receptor (TCR). In this respect a genetic linkage of TCR α/δ -complex to specific IgE response was reported by Moffat *et al.* [16]. The authors assumed that the locus in the TCR- α/δ region on chromosome 14 may determine specific IgE response. Although the findings have not yet been confirmed, the TCR repertoire could play a restrictive role in the recognition of allergenic peptides, which could additionally be combined with a restriction due to the HLA-haplotype. Regarding the latter, previous studies of atopic humans who were skin-test positive to pollen allergens have revealed significant association between particular HLA-D types and specific immune responsiveness toward each of several highly purified pollen allergens [17,18]. Secondly, other elements involved in the process leading to IgE production, such as the B-cell receptor [27], can act restrictively. As a result, monosensitization could occur in a subpopulation of atopic individuals. However, among a given population the majority shows production of IgE antibodies against more than one common inhalant allergen, which might be at least in part the consequence of crossreactivity of allergens.

Crossreactivity among pollen allergens at the antibody level has been considered a primary basis for years [19]. More recently, a growing body of literature about

crossreactivity at the level of T-cell recognition can be found. An allergen molecule usually encompasses different epitopes, which can be recognized by T cells. Some groups of allergens, such as those derived from pollens share some epitopes. Others might show little or no homology. Theoretically, a high level of homology of a group of allergens would result in crossreactivity on the T-cell level [20–22], and thus increases probability of IgE antibody production against the respective allergens. This could be an explanation for our finding that sensitization to hazel and birch pollens coincided (Table 4). The homology of tree and grass pollens has not yet been investigated. However, Mohapatra found restricted TCR V gene usage of epitopes from Lol p1 and Poa p IX. The author assumed that induction of cross-proliferative T cells could constitute the basis for the extensive crossreactivities at the antibody level, and that they were responsible for the induction and/or the development of concomitant sensitivity to several allergenic proteins [23].

Most mite-allergic individuals are sensitized to *Dermatophagoides pteronyssinus* (Dpt) and *Dermatophagoides farinae* (Df). A structural similarity of the allergens Der p1 and Der f1, which leads to crossreactive IgE production, has already been reported by Heymann *et al.* [24]. Common and species-specific epitopes had been defined on Der p1 and Der f1, and crossreactivity was demonstrated by polyclonal rabbit antibodies [25,26]. To the best of our knowledge no previous publication has reported homologies of allergens from different animal species, such as Fel d1 and Can f1. A negative association of birch pollens and cat dander might be explained as being due to different structural homologies of these allergens. But no causal relationship for the co-sensitization has been reported before.

For practical purposes our data might be relevant with respect to three situations: firstly, pet-owners sensitized to cat or dog dander should be recommended to give up their pet and not to switch to another pet, as they have a high risk of a co-sensitization to animal dander. Secondly, before starting immunotherapy, patients monosensitized to one kind of pollen should be informed that they have an increased risk for acquiring an additional sensitization to other pollens. Thirdly, the state of monosensitization to mite allergens should be used as an argument to convince the respective parents on the subject of allergen avoidance, as these measures may be particularly effective especially in monosensitized patients [4].

In summary, our data suggest that phenotypes of specific sensitization to common inhalant allergens in populations living in a given environment do not occur randomly. In particular, monosensitization to Dpt and combined sensitizations to pollen allergens and animal dander appear as frequent and distinct patterns. Monosensitization might be explained primarily by restrictive role, e.g. of the T-cell

receptor repertoire, whereas combinations of sensitization could be caused by the epitope sharing of highly homologous allergens. Thus, our data point out that experimental findings about antigen recognition and allergen structure might be linked to the atopic phenotype at the population level. Moreover, our findings regarding monosensitization and clustering of different sensitizations can be helpful when counselling and informing atopic patients.

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