
Age-dependent tendency to become sensitized to other classes of aeroallergens in atopic asthmatic children

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Background: Several longitudinal studies report that allergic sensitization increases with age from childhood to adulthood.

Objective: To evaluate whether an age-dependent tendency to become sensitized to new classes of allergens is present in atopic children, we studied retrospectively the changes in allergic sensitization in 165 asthmatic patients, monosensitized (ie, sensitized to only one class of allergens) in the first survey.

Methods: All the children (18 months to 8 years at enrollment), attended our outpatient clinics twice, at time intervals ranging from 2 to 10 years. On each visit, sensitization to house dust mites, pollens, animal danders, and molds was determined by skin prick test.

Results: We found that 43.6% ($n = 72$) of the patients became polysensitized on the second survey. According to age on first survey, the patients were further divided into two age groups: (1) group 1 = 18 months to <5 years old ($n = 98$) and (2) group 2 = 5 to 8 years ($n = 67$). The transition from monosensitization to polysensitization observed in the entire population was present in both groups: 47 (47.9%) of the 98 children in group 1 and 25 (37.3%) of the 67 children in group 2 showed to be sensitized to more classes of allergens, as compared with first survey. Both in the whole population and in the two age subgroups, the changes in the frequency of monosensitization between the two evaluations were time-dependent ($P < .05$, each χ^2). Finally, to investigate whether monosensitization to a specific class of allergens could favor the development of polysensitization, we evaluated the frequency of polysensitization in the second survey in patients originally monosensitized to house dust mites or to pollens. We found that of the 130 patients originally monosensitized to house dust mites, 59 became polysensitized (45.4%), while of the 28 patients originally monosensitized to pollens, 9 became polysensitized (32.1%) ($P > .1$). Similar results were obtained when patients were divided into age groups.

Conclusion: These data demonstrate that (1) monosensitized children are likely to become polysensitized and (2) house dust mite sensitization and, at a lower degree, pollen sensitization, apparently seem to play a "triggering" role in the development of polysensitization, since a high proportion of children originally monosensitized to house dust mites or to pollens became polysensitized.

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INTRODUCTION

Over the last 20 to 30 years, the prevalence of atopic diseases has increased

considerably in developed countries. Population-based studies showed this was true at least in children and young adults.¹⁻⁷ Atopic diseases are complex and multifactorial since their development and phenotypic expression depend on interaction between genetic factors, environmental exposure to allergens, and nonspecific adjuvant factors, such as tobacco smoking, air pollution, and infections.⁸ The relative roles of genetic and environmen-

tal factors in allergic diseases have not been completely clarified yet.⁹⁻¹² Subjects genetically prone to sensitization are likely to require prolonged exposure to allergens before developing respiratory allergic symptoms. In this context, several longitudinal studies in birth cohorts and in subjects with asthma report that allergic sensitization persists¹³ and increases with age from childhood to adulthood, reaching its peak in early adulthood.¹⁴⁻¹⁶ Although some factors facilitating the development of allergy have been identified,¹⁷⁻²¹ the development of allergic sensitization in children and the factors that may influence sensitization to one rather than to more different types of allergens are still not completely known. Specifically, little is known about the longitudinal intraindividual change in number and type of specific allergens from early childhood to adolescence. In this context, a previous study²² reported that the percentage of children sensitized to only one class of allergens decreased with age of the subjects compared with polysensitized subjects. Particularly, a more pronounced decrease in the frequency of monosensitization was observed in children younger than 7 years.²² To the best of our knowledge, no study has been published to date evaluating how and to what extent monosensitization varies with time. The aim of this study was therefore to investigate the time-dependent changes in sensitization to different airborne allergens in a sample population of monosensitized preschool and school children.

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MATERIALS AND METHODS

Study Design

We retrospectively analyzed the records of 343 consecutive children, aged between 18 months and 8 years old, who attended twice as outpatients the Pediatric Department or the Pulmonary Division of the Gaslini Institute (Genoa, Italy) for clinical manifestation of bronchial asthma; these patients were referred to our departments by their primary care physicians. We tested these patients by skin prick test with the four most common classes of aeroallergens (see below for details) and we found that 74 (21.6%) were negative, while the remaining 269 were skin prick test positive. Out of the skin prick test positive patients, 104 (38.7%) were polysensitized and the remaining 165 (61.3%) were monosensitized. This latter group of 165 monosensitized asthmatic subjects (male to female ratio = 1.38) was included in the present paper, whereas the analysis of data on the negative and the polysensitized children will be a part of a separate study. Asthma was defined on the basis of clinical manifestations, according to the guidelines proposed by the Medical Section of the American Lung Association.²³ In the first survey, all the children aged between 18 months and 8 years were monosensitized, ie, sensitized to only one class of allergens; positive skin test results to several pollens were classed as monospecific. The time interval between the two visits ranged from 2 to 10 years. Each visit included skin prick tests and patient evaluation including case history and physical examination. According to age in the first survey, the children were divided into preschool children (ie aged 18 months to <5 years, n = 98, 59.4%) and school children, aged 5 to 8 years (n = 67, 40.6%). All of them lived in Genoa, a city on the Northwestern sea coast of Italy. Verbal informed consent was obtained by parents.

Skin Prick Test Procedure

Sensitization to the four most common classes of aeroallergens was evaluated by skin prick test.²⁴ The allergen pan-

Table 1. Prevalence of Allergen-Specific Monosensitization in the Entire Population and in each Age Group on the First Survey

	Entire Population		Group 1 18 mo to <5 years old		Group 2 5 to 8 years old	
	No	%	No	%	No	%
House dust mites	130	78.8	80	81.6	50	74.6
Pollens	28	17	13	13.3	15	22.4
Pets	5	3	4	4.1	1	1.5
Molds	2	1.2	1	1	1	1.5
All allergens	165	—	98	—	67	—

els tested included (1) house dust mite class (*Dermatophagoides pteronyssinus* 5,000 PNU/mL and *Dermatophagoides farinae* 5,000 PNU/mL); (2) pollen class (*Parietaria officinalis* 1,000 UP/mL, mix of Graminae 10,000 UP/mL, Compositae 10,000 UP/mL, Betulaceae 10,000 UP/mL, Oleaceae 10,000 UP/mL), (3) pet dander class (cat and dog skin scale allergen extracts 1:20) and, (4) molds (mix of *Aspergillus* 10,000 PNU/mL, *Cladosporium* 10,000 PNU/mL, *Alternaria tenuis* 10,000 PNU/mL), (Bayropharm, Milan, Italy). A histamine solution in distilled water (10 mg/mL) was used as positive control and the glycerol-buffer diluent of the allergen preparations as negative control. Each subject was skin tested in duplicate on the volar surface of the forearm using 1-mm prick lancets (Dome/Hollister-Stier, Great Britain). On each visit, skin prick test was performed using the same panel of allergens. The tests were carried out by two specially trained nurses and the wheal reaction read by the same nurses under the supervision

of a physician. The reactions were recorded within 15 minutes by evaluating the skin response rate to the inoculation of each allergen in comparison to the wheal given by the negative control. A wheal diameter 3 mm larger than the negative control was considered a positive reaction. Long-acting beta₂ adrenoreceptor agonists and antihistaminic preparations were stopped 3 weeks before skin testing.

Statistical Analysis

Prevalence rates were calculated; chi² test or chi² test for trend were used to compare the rates of each group. *P* values ≤ .05 and ≤ .01 were considered, respectively, as statistically significant and highly statistically significant.

RESULTS

The prevalence of allergen-specific sensitization in the entire population and in each age group respectively in the first and second surveys is illustrated in Tables 1 and 2. As expected, allergy to house dust mites was the

Table 2. Prevalence of Allergen-Specific Sensitization in the Entire Population and in each Age Group on the Second Survey

	Entire Population		Group 1 18 mo to <5 years old		Group 2 5 to 8 years old	
	No	%	No	%	No	%
House dust mites	136	82.4	84	85.7	52	77.6
Pollens	82	49.7	50	51	32	47.8
Pets	34	20.6	23	23.4	11	16.4
Molds	11	6.7	6	6.1	5	7.5
All allergens	165	—	98	—	67	—

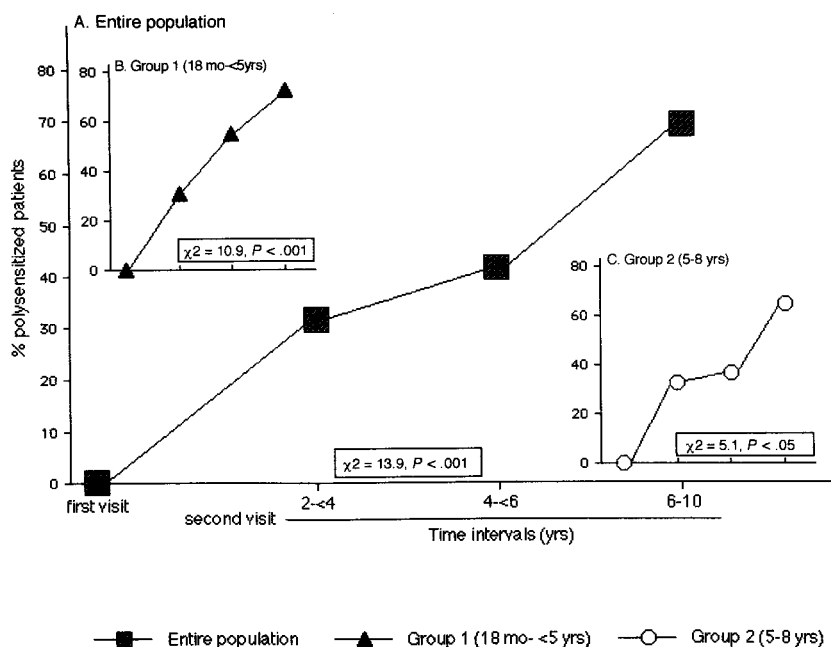


Figure 1. Changes in the frequency of polysensitization with time. On the abscissa, the percentage of polysensitized children is indicated; on the ordinate, the different time intervals between the two visits expressed in years are shown. χ^2 test for trend was used for statistical analysis. ■ = entire population, ▲ = children aged between 18 months and <5 years old; ○ = children aged between 5 and 8 years.

most prevalent sensitization followed by allergy to pollens, pets and molds, both in the monosensitized group on the first survey and in the monosensitized + polysensitized group in the second survey (Tables 1 and 2). No reversions from positive to negative skin reactions from the first to the second surveys were recorded.

The evaluation of the changes in the frequency of monosensitization from the first to the second survey demonstrated that 72 (43.6%) of 165 originally monosensitized children became polysensitized. Similar results were obtained analyzing separately pre-school and school children: 47 (47.9%) of the 98 children in group 1 and 25 (37.3%) of the 67 children in group 2 showed new sensitizations as compared with those observed in the first survey.

To evaluate whether the changes in the frequency of monosensitization were time-dependent, we evaluated the prevalence of monosensitization in subjects who attended our labs for the second evaluation (1) 2 to <4 years,

(2) 4 to <6 years, (3) 6 to 10 years after the first visit. We found that the percentage of children still sensitized to only one class of allergens became

lower as the time interval between the two visits increased ($\chi^2 = 13.9, P < .001$) (Fig 1). Similar results were obtained analyzing separately the two age groups (preschool children: $\chi^2 = 10.9, P < .01$ and school children: $\chi^2 = 5.1, P < .05$), (Fig 1).

To investigate whether monosensitization to a specific class of allergens could be related to the subsequent development of polysensitization, we evaluated the frequency of polysensitization on the second survey in children originally sensitized only to house dust mites or only to pollens. We found that, out of the 130 patients originally monosensitized to house dust mites, 59 became polysensitized (45.4%), while of the 28 children originally monosensitized to pollens, 9 became polysensitized (32.1%; $\chi^2 = 1.2, P > .1$), (Fig 2). Similar results were obtained investigating pre-school children ($\chi^2 = 0.15, P > .1$; Fig 3A) and school children ($\chi^2 = 0.4, P > .1$; Fig 3B). Although there was a slightly higher tendency of children monosensitized to house dust mites to become polysensitized (as compared with children monosensitized to pollens), the difference was not statistically significant.

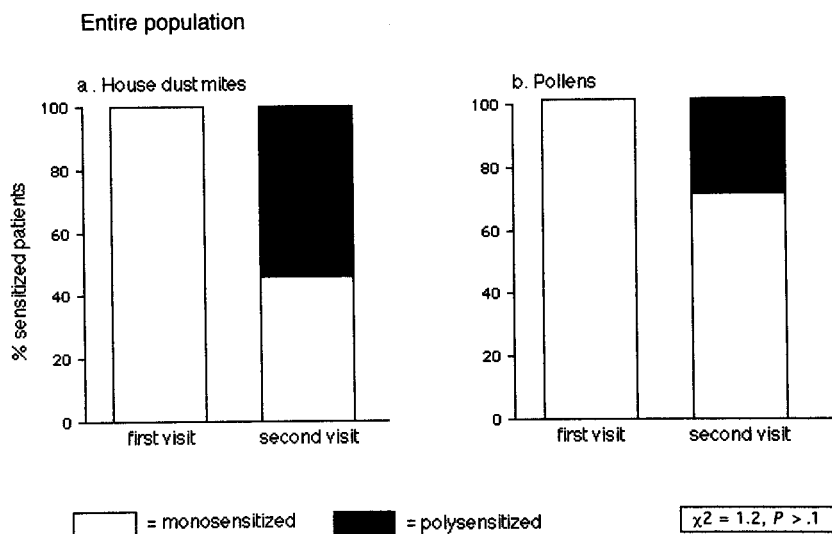
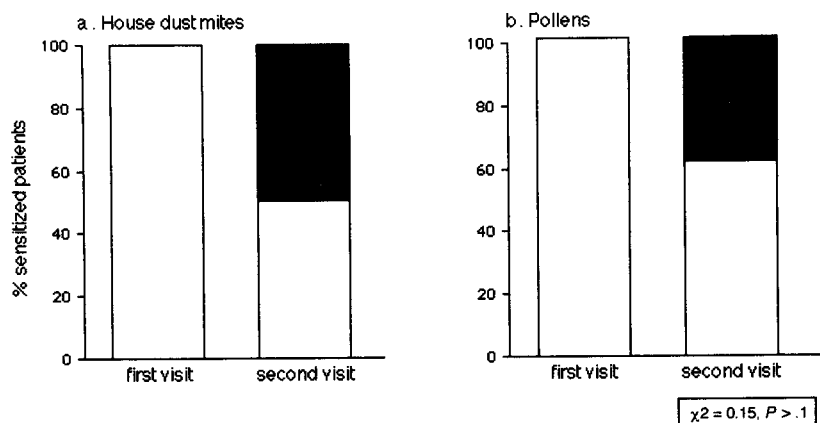
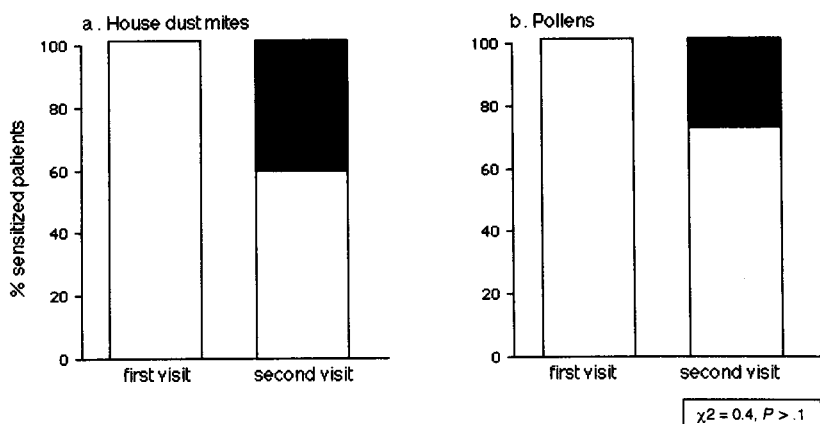


Figure 2. Frequency of polysensitization on the second survey in children originally monosensitized to house dust mites (a) or to pollens (b) evaluated by skin prick test. On the abscissa, the two surveys are indicated; on the ordinate, the percentages of monosensitized (□) or polysensitized (■) children are described.

A. Group 1 (18mo-<5 yrs old)



B. Group 2 (5-8 yrs old)



□ = monosensitized ■ = polysensitized

Figure 3. Frequency of polysensitization on the second survey in children originally monosensitized to house dust mites (a) or to pollens (b) evaluated by skin prick test. Panel A = children aged between 18 months and <5 years old; Panel B = children aged between 5 and 8 years old. On the abscissa, the two surveys are indicated; on the ordinate, the percentages of monosensitized (□) or polysensitized (■) children are described.

DISCUSSION

The main findings of the present study on asthmatic children are the following: (1) the vast majority of monosensitized children showed skin reactivity to house dust mites, (2) a high proportion of individuals who were originally monosensitized became polysensitized and this proportion increased with time, and (3) a high proportion of chil-

dren originally monosensitized to house dust mites or to pollens became polysensitized.

The group of individuals studied included children with asthma, evaluated as "out patients" in the Pediatric Department or in the Pulmonary Division of the Giannina Gaslini Institute in Genoa, a city located on the North Western sea coast of Italy. Even if the

group of children evaluated is not representative of the general population, it represents the Ligurian children who need medical care for respiratory symptoms.

Among monosensitized children, the evaluation of the frequency of skin reactivity to the various classes of allergens (house dust mites, pollens, pets, and molds) demonstrated that monosensitization to house dust mites was the most frequent occurrence, followed by sensitization to pollens, pets, and molds. Different hypotheses may explain these results. Taking into account that several studies demonstrated that the immune response of patients allergic to only one allergen seems to differ from that of subjects allergic to multiple allergens,²⁵⁻²⁷ a high "sensitization threshold" in monosensitized subjects could be hypothesized. In other words, monosensitized subjects seem to require a stronger exposure to the sensitizing allergen and, as a consequence, monosensitized individuals will probably develop more frequently allergy to a perennial and ubiquitous allergen like house dust mites, rather than to seasonal allergens. In addition, house dust mites seem to be able to stimulate the immune system early in life, as reported by Mariani et al who found IgG1 subclass antibodies against *Dermatophagoides pteronyssinus* in the serum of subjects aged 1 year.²⁸

Evaluating the skin prick test reactions from the two surveys, no reversions from positive to negative were recorded. This observation is similar to that reported by Barbee et al on a community sample population. Briefly, the authors evaluated a cohort of 1333 subjects aged ≥ 3 years followed for a mean of 8.1 years to assess changes in allergen skin test reactivity: they found that there was no lost of sensitization among sensitized subjects younger than 15 years.¹⁴

We also found that a high percentage of monosensitized children tended to become polysensitized and that sensitization to only one class of allergens decreased with time as polysensitization increased. Since exposure to new allergens is likely to increase with

time, it could be hypothesized that children originally sensitized to only one class of allergens may develop sensitization to newer allergens encountered in the course of their life. Is there any allergen able to "prime" the sensitization to another allergen? To answer this question, an in depth investigation of various risk factors is required. Nonetheless, we noticed that over 40% of children originally monosensitized to house dust mites and about 30% of these originally monosensitized to pollens became polysensitized. Then, house dust mite sensitization and, to a slightly lower degree, pollen-sensitization, apparently seems to play a "triggering" role in the development of polysensitization, even if this observation was not confirmed by statistical analysis.

Our results are partially supported by a recent study by Koh et al.²⁹ who reported that early-phase and late-phase reactions to German cockroach challenge were augmented by the preceding reaction to *Dermatophagoides pteronyssinus*. This suggests that a preceding airway response to one allergen may lead to priming with enhancement of the early and late airway responses to the subsequent challenge with another allergen.²⁹ The mechanism underlying priming by one allergen of another allergen is not clear. The finding that this possible "triggering" role in the development of further sensitizations could be played by house dust mites, and to a lower degree by pollens, could be explained by the clinical observation that asthmatic patients allergic to both pollen and a second allergen are more prone to asthmatic symptoms on contact with the second allergen during the pollen season than during the rest of the year.³⁰ Taking into account that exposure to house dust mites is not only more prolonged than that to pollens, but also perennial and ubiquitous, it could be hypothesized that asthmatic patients allergic to house dust mites may be more prone to develop further sensitization than those sensitized to pollens. To better prove this finding, it would be very interesting to investigate genetic and environ-

mental risk factors able to influence the development of polysensitization towards the different airborne allergens in a larger sample population of children monosensitized to house dust mites or to pollens.

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