

Relationships of allergic sensitization, total immunoglobulin E and blood eosinophils to asthma severity in children of the EGEA Study

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Summary

Background Although allergy is highly associated with childhood asthma, it is not well known if there is a relationship between the intensity of allergic sensitization and asthma severity.

Objective The objectives of the study were to examine the relationships between several markers of allergy and asthma severity in asthmatic children included in the Epidemiological study on the Genetics and Environment of Asthma, bronchial hyper-responsiveness and atopy (EGEA).

Methods The population comprised 216 asthmatic children below 16 years of age. Total IgE and blood eosinophil counts were measured and skin prick tests to 11 aeroallergens were performed. The intensity of the allergic sensitization was assessed by the number of positive skin prick tests and by skin weal sizes. Asthma severity was measured with four criteria: a clinical severity score, history of hospitalization for asthma, FEV₁% predicted and inhaled steroid use in the last 12 months.

Results Most of the children were sensitized to at least one aeroallergen (88.2%). Atopy was not related to the severity of asthma, except for a tendency for a more severe clinical score in non-atopic children. The type and intensity of the allergic sensitization were not associated with any criteria of asthma severity. Total IgE was significantly increased in children treated with inhaled corticosteroids and in children ever hospitalized for asthma (*P*-values 0.009 and 0.04, respectively). Eosinophil counts were not related to asthma severity.

Conclusion Our results suggest that severe childhood asthma may be related to a high level of total IgE but not to blood eosinophil counts. The lack of positive relationships between both atopy and the intensity of allergic sensitization with asthma severity supports the hypothesis of different risk factors being associated with asthma and with the severity of asthma.

Keywords asthma, atopy, children, eosinophils, IgE, severity

Submitted 2 July 2002; revised 10 September 2002; accepted 6 February 2003

Introduction

Asthma is a disease of variable severity. Patients with severe and/or uncontrolled asthma are at greater risk of death, are more likely to require emergency visits and hospital admissions, and report worse quality of life. Severe asthma in children tends to persist in adulthood. Therefore the understanding of risk factors for asthma severity has important implications for treatment and prevention. Allergic reactions are major causes of airway inflammation, which is a cornerstone of asthma severity [1].

Asthma in childhood is associated with markers of allergy. Total IgE levels are associated with physician's diagnosis of asthma and the presence of bronchial hyper-responsiveness in children [2]. Increased IgE may be a risk factor for the later development of asthma in childhood [3]. Blood eosinophils are elevated in children with asthma [4]. Specific sensitization to

aeroallergens and especially to *Dermatophagoides pteronyssinus*, *Aspergillus* and cat is strongly associated with current asthma in cross-sectional studies in children [5–9]. Furthermore, Sears et al. have shown a significant dose–response relationship between the number of positive skin prick tests and the prevalence of asthma symptoms [6]. In general, factors related to the occurrence of the disease may not be the same as those related to the severity of the disease and results have already shown that this is the case for asthma [10, 11].

Few studies have examined the relationships of allergy markers with asthma severity in children. Some studies showed a worse asthma severity with sensitization to aeroallergens [12, 13] but others did not [14, 15]. Blood eosinophils are related to asthma severity in adults [16]. In children there are conflicting results regarding the relationships of asthma severity with both blood eosinophils [4, 17] and serum total IgE [13, 18].

The objectives of this study were to examine the relationships between biological markers of allergy (total IgE, blood eosinophil counts, intensity and type of allergic sensitization) and asthma severity in asthmatic children included in the EGEA study (Epidemiological study on the Genetics and Environment of Asthma, bronchial hyper-responsiveness and atopy).

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Methods

EGEA is a case-control and family study of adult and childhood asthma [19]. Briefly, asthmatic cases were recruited in outpatient chest clinics in five French cities if they had a positive answer to four validated and standardized questions of a questionnaire completed by adults or by parents of children: (1) Have you (or your child) ever had attacks of breathlessness at rest with wheezing? (2) Have you (or your child) ever had asthma attacks? (3) Was this diagnosis confirmed by a physician? (4) Have you (or your child) had asthma attacks in the last 12 months? First-degree relatives of children and adult cases were invited to participate in the EGEA study, but only asthmatics were included in the present analysis. In relatives, asthma status was based on a positive answer to the first or the second question. Totally, the study population comprised two groups of asthmatic children below 16 years of age: 135 cases recruited in outpatient chest clinics and 82 asthmatic relatives of cases not necessarily followed-up in chest clinics.

Parents answered a questionnaire about their child respiratory status, including detailed questions on asthma. Children above 7 years of age performed forced expiratory manoeuvres with a nose clip to measure FEV₁. Participants were instructed not to use β -agonists within 6 h of the test. Skin prick tests (SPT) to 11 allergens (cat, *Dermatophagoides pteronyssinus*, *Cladosporium herbarum*, *Alternaria tenuis*, timothy grass, olive, birch, *Parietaria judaica*, *Aspergillus*, ragweed and *Blattella germanica*) as well as negative and histamine positive controls were performed in children over 4 years of age on the forearm. Positive SPT were defined by a mean weal diameter ≥ 3 mm larger than the negative control at 15 min after application and atopy by the presence of at least one positive SPT. The weal size of positive responses, and the number of positive skin tests were also studied. Polysensitization was defined by the presence of at least two positive SPT. According to the type of allergens, three groups of allergic sensitization were defined: sensitization to indoor allergens (cat, *Dermatophagoides pteronyssinus* or *Blattella germanica*), sensitization to outdoor allergens (timothy grass, olive, birch, *Parietaria judaica* or ragweed) and to moulds (*Cladosporium herbarum*, *Alternaria tenuis*, *Aspergillus*). Atopy, polysensitization and type of sensitization were analysed only in children who had performed all skin prick tests and who had a positive test to histamine (122 cases and 73 relatives).

Total IgE was measured by radioimmunoassay (Phadebas PRIST technique; Pharmacia Diagnostics, AB, Lyon, France). All determinations were performed in one central laboratory (Institut Pasteur, Lyon, France). Eosinophil counts were performed on peripheral blood and expressed as absolute numbers or percentages. The results were similar when blood eosinophil counts were analysed as absolute numbers or as percentages. Therefore only the results of the absolute numbers are shown.

The choice of items used to assess asthma severity was based on international guidelines and was previously used [10]. It allowed us to approach several dimensions of the severity. (1) A composite clinical score ranging from 0 to 7 including symptoms of the last 12 months: asthma attack frequency (from 0 for less than once a month to 3 for at least once a day), persisting symptoms between attacks (from 0 for none to 3 limiting activities), and hospitalization for asthma in the last

12 months (0 for none, 1 for more than 0), used both as a continuous variable or as a binary response, mild (score < 2), severe (score ≥ 2). (2) Hospitalization for asthma at any time in life. (3) A measure of ventilatory function: FEV₁ in percentage predicted (used as a continuous variable or as dichotomous < 80% and $\geq 80\%$). (4) A therapeutic item: report of inhaled corticosteroids use in the last 12 months.

Statistical analysis

Relationships between allergy and asthma severity were analysed using successively different markers of allergy: first two biological markers (total IgE, blood eosinophil counts), then the presence of positive SPT to aeroallergens, the degree of sensitization measured by weal sizes of the skin tests and finally polysensitization expressed as the number of positive skin tests. Total IgE and blood eosinophil counts were log transformed and results expressed as geometric means (GM). Due to the familial aggregation of the data, generalized estimated equations (GEE) were performed to take into account dependence between observations [20]. Using the statistical software package SAS, the GENMOD procedure was used for categorical response variables, and MIXED procedure for continuous response variables.

Results

The main characteristics of the population studied are shown in Table 1. The mean age of the children was 11 years (range 2.7 to 16.4) and there were more boys than girls (Table 1). As expected by the protocol, cases recruited in chest clinics had more severe asthma than relatives not followed-up in specialized chest clinics (Table 1). Thus, the range of asthma severity was wide, as shown by the description of asthma severity items in the population, with more than half with less than one asthma attack per month and approximately a third with a severe clinical score (Table 1). Items used to assess asthma severity were significantly related, except that FEV₁ was neither associated with inhaled corticosteroid treatment nor with the clinical score (results not shown). Most children were sensitized to at least one aeroallergen (88.2%) and 68.6% of sensitized children had a polysensitization defined by the presence of more than one positive SPT (Table 1). The four more frequent allergic sensitizations were developed towards *Dermatophagoides pteronyssinus* (67.7%), timothy grass (36.9%), cat (25.6%) and *Blattella germanica* (25.1%). Few children were sensitized to outdoor allergens only (4.6%) or moulds only (2.6%), so analyses regarding the potential effect of the type of sensitization were restricted to children sensitized to indoor allergens only (34.4%), to outdoor allergens and/or moulds (9.2%) and to both categories (44.6%). Cases had higher total IgE and blood eosinophil counts than relatives (Table 1).

Total IgE level was significantly increased in children treated with inhaled steroids in the last 12 months and in children ever hospitalized for asthma (Table 2), but not in children hospitalized in the last 12 months ($P = 0.86$). Clinical severity score and FEV₁, examined either as binary or as continuous variables, were not associated with total IgE. Pearson correlation coefficients calculated on continuous variables were 0.09 ($P = 0.16$) for the clinical severity score and 0.11 ($P = 0.12$) for FEV₁.

Table 1. Descriptive characteristics of asthma and allergy in asthmatic children (cases and relatives of cases)

	Cases	Asthmatic relatives	All
<i>n</i>	135	82	216
Age, mean $\pm$ SD	11.2 $\pm$ 2.1	10.6 $\pm$ 3.7	11.0 $\pm$ 2.8
Sex, % boys	68.9	55.6	63.4
Asthma severity criteria			
Frequency of asthma attacks			
$\leq$ 1/month, %	45.2	71.0	53.9
$>$ 1/month to $<$ 1/week, %	37.0	20.3	31.4
$\geq$ 1/week, %	17.8	8.7	14.7
Severe clinical score, %	33.3	26.1	30.8
Hospitalization ever, %	37.8	10.3	28.6
FEV ₁ $\leq$ 80% predicted, %	17.2	13.2	15.8
Inhaled steroid (last 12 months), %	58.3	15.0	42.0
IgE IU/ml, GM [95% CI]	402 [45–3597]	246 [13–4670]	337 [27–4204]
Eosinophils nb/mm ³ , GM [95% CI]	437 [120–1595]	384 [70–2117]	417 [97–1792]
Sensitization, <i>n</i> *	122	73	195
Sensitization to specific allergens (SPT)			
<i>Dermatophagoides pteronyssinus</i> , %	76.2	53.4	67.7
Timothy grass, %	36.1	38.4	36.9
Cat, %	32.0	15.1	25.6
<i>Blattella germanica</i> , %	23.8	27.4	25.1
<i>Alternaria tenuis</i> , %	16.4	19.2	17.4
Birch, %	9.8	13.7	11.3
Olive, %	6.6	17.8	10.8
<i>Aspergillus</i> , %	3.3	8.2	5.1
<i>Cladosporium herbarum</i> , %	2.5	2.7	2.6
Ragweed	2.5	2.7	2.6
<i>Parietaria judaica</i> , %	1.6	1.4	1.5
Sensitization to any allergen, % (<i>n</i>)	92.6 (113)	80.8 (59)	88.2 (172)
In sensitized children,			
Number of positive SPT, mean $\pm$ SD	2.3 $\pm$ 1.1	2.5 $\pm$ 1.5	2.3 $\pm$ 1.2
$\geq$ 2 positive SPT, %	71.7	62.7	68.6

*Children with a positive skin test to histamine and all skin tests performed. GM, geometric mean.

Table 2. Relationships of asthma severity with total IgE and blood eosinophils (absolute numbers)

	IgE, GM	<i>P</i>	Eosino nb, GM	<i>P</i>
Clinical severity score				
Mild	342	0.68	411	0.36
Severe	372		458	
Hospitalization ever				
No	317		419	
Yes	472	0.04	446	0.57
FEV ₁ (% predicted)				
$>$ 80%	369		405	
$\leq$ 80%	286	0.30	457	0.39
Inhaled steroids (last 12 months)				
No	271		419	
Yes	435	0.009	415	0.93

GM, geometric mean.

Eosinophil counts were not related to any asthma severity criteria studied (using both continuous and binary variables to express FEV₁ and clinical severity score). Thirty percent of the

population had taken oral corticosteroids in the last 12 months but no information on the date of the intake was available. Due to the known effect of oral corticosteroids on blood eosinophils, adjustment on treatment with oral corticosteroids was performed and did not change the results.

Atopic children had a significantly milder asthma compared with non-atopic children, with mean severity scores of 1.1 vs. 1.9 in atopics and non-atopics, respectively ($P=0.02$). Using the binary response for the clinical severity score, OR [95% C.I.] associated with atopy was 0.29 [0.10; 0.79] (Table 3). The frequency of atopy was significantly lower in children hospitalized in the last 12 months than in non-hospitalized children (69.2% vs. 91.1%, OR [95% C.I.] = 0.22 [0.06; 0.80]). Sensitizations to *Dermatophagoides pteronyssinus* and cat were significantly related to a lower clinical score (Table 3) but the differences did not reach significance when analysing the clinical severity score as a continuous variable (mean severity scores in sensitized and non-sensitized children to *Dermatophagoides pteronyssinus* were 1.38 vs. 1.05 ($P=0.14$) and to cat 1.25 vs. 0.88 ($P=0.10$), respectively). The clinical severity score was not related to a positive SPT to *Alternaria tenuis* or *Blattella germanica*. Inhaled steroid treatment and low FEV₁ were not associated with the presence of atopy or with a positive SPT to any of the four specific allergens studied. Children who had ever

Table 3. Relationships between sensitization to at least one allergen or specific allergens and asthma severity

	Percentage of children sensitized to			
	≥ 1 allergen	Der P	Cat	<i>Alternaria tenuis</i>
Clinical severity score				
Mild	94.2	76.7	31.7	19.1
Severe	82.3	56.9	17.7	13.7
P	0.02	0.004	0.06	0.46
Hospitalization ever				
No	90.8	74.6	27.7	16.9
Yes	87.0	61.1	25.9	18.5
P	0.45	0.07	0.73	0.81
FEV ₁ (% predicted)				
> 80%	89.3	70.4	25.8	17.6
≤ 80%	86.2	65.5	31.0	17.2
P	0.61	0.57	0.55	0.99
Inhaled steroids (last 12 months)				
No	85.8	64.6	22.1	14.1
Yes	91.2	72.5	31.2	21.2
P	0.21	0.24	0.14	0.19

Der P, *Dermatophagoides pteronyssinus*.

Table 4. Relationship between the degree of sensitization and asthma severity in sensitized children

	Number of positive SPT	Der P weal size (mm)	Cat weal size (mm)	<i>Alternaria</i> weal size (mm)
n	172	132	50	34
Clinical severity score				
Mild	2.4	5.8	4.4	5.3
Severe	2.3	6.3	3.8	4.2
P	0.77	0.40	0.27	0.13
Hospitalization ever				
No	2.4	5.8	4.4	5.1
Yes	2.4	6.5	3.9	5.6
P	0.90	0.14	0.24	0.48
FEV ₁ (% predicted)				
> 80%	2.4	6.0	4.3	5.5
≤ 80%	2.4	6.1	4.2	4.0
P	0.91	0.77	0.77	0.09
Inhaled steroids (last 12 months)				
No	2.3	5.8	4.1	5.5
Yes	2.5	6.2	4.4	4.9
P	0.32	0.28	0.43	0.37

Der P, *Dermatophagoides pteronyssinus*.

been hospitalized for asthma tended to be less sensitized to *Dermatophagoides pteronyssinus*, but the association was only of borderline significance ($P = 0.07$). Timothy grass sensitization was not related to any asthma severity criteria.

In sensitized children, asthma severity was not related to the type of allergic sensitization (indoor vs. outdoor/moulds) (results not shown). The number of positive SPT was not associated with any of the four asthma severity criteria studied

(Table 4). This was also the case considering skin weal sizes to *Dermatophagoides pteronyssinus*, cat and *Alternaria tenuis*.

Discussion

In the asthmatic children from the EGEA study exhibiting a wide range of asthma severity, total IgE was positively associated with hospitalization ever for asthma and inhaled steroid treatment in the last 12 months. The lack of positive relationships between asthma severity and blood eosinophil counts and the presence or intensity of allergic sensitization is not in favour of a major role for these markers on asthma severity.

The present analysis was performed in a large population of well-characterized asthmatic children. It included child cases recruited in chest clinics, therefore mostly severe (for example 17.8% had more than one asthma attack per week), and asthmatic relatives of cases, mostly mild (for example 71% had less than one asthma attack per month). The same analyses restricted to asthmatic cases, a more homogeneous population, showed similar results. The negative findings are not explained by the lack of statistical power. For continuous outcome variables the study had a 80% power to detect an effect size of 0.47 for the severity score, 0.57 for FEV₁, 0.41 for ICS treatment and 0.45 for hospitalization. For example, this corresponds for eosinophils to a power sufficient to find a significant difference if severe asthmatics had 581 eosinophils/mm³ whereas the mild ones had 411 eosinophils/mm³. There is no accepted epidemiological definition of asthma severity in children. The criteria used to assess asthma severity have been widely used in the literature. They are meant to cover different dimensions of the severity [21] that are not always correlated together [22, 23]. Hospitalization ever for asthma and inhaled corticosteroid use may be considered as severity markers, as opposed to recent symptoms and FEV₁, which may reflect the control of the disease [24].

The relationship between total IgE and asthma prevalence is well known [2]. Longitudinal studies suggest a prognostic value of total IgE for the severity and persistence of BHR [17, 25] or the persistence of wheeze after 11 years of age [26, 27]. In a general population sample of 562 children, Sears et al. showed that the presence and the degree of airway hyper-responsiveness were related to total IgE in a cross-sectional study [2]. However Takeda et al. did not find such a relationship in a small sample of 47 asthmatic children [18]. Some studies have suggested a role of total IgE in asthma severity as expressed by the occurrence of asthma exacerbation in young children [13], or an asthma severity score based on frequency of asthma attacks and asthma treatment [28]. In children recruited in the EGEA study, total IgE level was significantly higher in inhaled steroid-treated children and in children ever hospitalized for asthma but was not associated with the clinical severity score or FEV₁. Our results seem to confirm the relationship between total IgE and asthma severity, but it seems less obvious that it is associated with asthma control.

Few studies have examined the relationship between blood eosinophils and asthma severity in children. Studies have shown positive correlations between blood eosinophil counts and the intensity of asthma symptoms [4, 28] and negative correlations with FEV₁ [4, 29]. However, eosinophil counts were not related to the severity and change over time of bronchial

responsiveness in a study following asthmatics from childhood to adulthood [17]. None of the asthma severity criteria studied in our analysis was related to blood eosinophils even when corticosteroid treatment was taken into account. This unexpected result may be explained because blood eosinophils do not exactly reflect eosinophilic airway inflammation [30] and/or neutrophils rather than eosinophils may be the predominant cells in severe asthma [31].

In EGEA, asthma severity was mostly independent of the presence of an allergic sensitization, even with a tendency towards less atopy in more severe asthma. Similar results have been found in the adult asthmatic population in the EGEA study [32] as well as in other studies of asthmatic adults [33]. In children, there are several results in the literature demonstrating a strong association between sensitization to aeroallergens and asthma prevalence [5, 6, 9]. But few studies have focused on the relationships between specific sensitization and asthma severity. In other studies examining children recruited in chest clinics, specific sensitizations to aeroallergens were not associated with several markers of asthma severity [14, 34]. A recent study combining a population-based sample and a hospital-based sample showed discordant results, i.e., the proportion of children with atopy increased with increasing wheeze frequency, but the sample included a large proportion of non-wheezing children (probably non-asthmatics) with a low prevalence of atopy [12]. In the several studies that showed an increased frequency of atopy in children hospitalized for asthma, it is worth noting that the analysis included control groups without asthma [35, 36]. Under the hypothesis that risk factors implicated in asthma are not necessarily those implicated in asthma severity the conclusion of studies including non-asthmatics may be more driven by asthma than by asthma severity. Discordant results regarding the relationship between asthma severity and atopy may be due to examination of different populations.

A higher prevalence of asthma has been found in subjects sensitized to indoor allergens, especially to *Alternaria* [6, 8]. Furthermore, sensitization to *Alternaria* has been associated with asthma severity in a general population of asthmatic adults and appeared a risk factor for severe asthma attacks in children and young adults [37, 38]. Results in EGEA did not confirm a positive relationship between sensitization to indoor allergens, to *Alternaria* or to cockroach and asthma severity. The level of exposure to allergens probably plays a role in determining the intensity of asthma symptoms or healthcare utilization, especially in sensitized children [14, 39]. The levels of exposure to specific allergens were not directly measured in EGEA. Therefore we cannot assess if the lack of association between specific sensitization and asthma severity in our study might be due to reduced exposure in more severe patients.

The degree of allergic sensitization (number and/or size of positive SPT) has been shown to be associated with the prevalence of asthma and bronchial hyper-responsiveness [6, 25, 40]. Previous studies showed linear relationships between dust mite weal sizes and asthma severity in a large population-based study and in a population-based sample enriched with children attending the emergency room for asthma [12, 41]. In 139 asthmatic children recruited in outpatient clinics, asthma severity evaluated by FEV₁ and medication usage was related to the number of positive SPT. In that study only children with the highest number of positive SPT had a significant increased risk

of severe asthma [15]. Our results did not show such a relationship, either using the number of positive skin tests or the size of the skin weal reactions to house dust mite, cat or *Alternaria* allergens. Further studies on population-based asthmatics, including a sufficiently large number of severe cases, should be undertaken. The presence or the intensity of allergy, which are positively associated with asthma, do not seem to be related to asthma severity in EGEA. It is of interest to note that a factor highly related to asthma such as allergy may not be related to asthma severity. Results regarding other factors related to asthma occurrence and asthma severity suggest that they may be different. Previous analyses in EGEA showed that active smoking was related to asthma severity but not to asthma occurrence [11], and that the severity of asthma in cases was not related to asthma occurrence in relatives but was related to asthma severity in asthmatic relatives [10]. These results support the hypothesis that different environmental and genetic risk factors are involved in asthma and in asthma severity.

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Data management INSERM ex-U155: J. Hochez. INSERM U472: N. Le Moual.

Acknowledgements

Supported in part by convention Institut National de la Santé et de la Recherche Médicale (INSERM)-Merk Sharp and Dohme, the INSERM networks of clinical research (489012) and public health research (493009), Ministry of Health, Direction de la recherche clinique CHU Grenoble and COMARES.

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