

Interrelationships of quantitative asthma-related phenotypes in the Epidemiological Study on the Genetics and Environment of Asthma, Bronchial Hyperresponsiveness, and Atopy

Marie-Pierre Oryszczyn, BSc,^{a,b} Emmanuelle Bouzigon, MD, PhD,^{c,d} Jean Maccario, PhD,^{a,e} Valérie Siroux, PhD,^f Rachel Nadif, PhD,^{a,b} Anne Wright, PhD,^g and Francine Kauffmann, MD^{a,b} Villejuif, Evry, Paris, and Grenoble, France, and Tucson, Ariz

Background: Delineating asthma subphenotypes is of interest to understand the cause of the disease. Few studies have addressed the interrelationships of quantitative asthma-related traits.

Objective: We sought to study the interrelationships of allergy markers and FEV₁ in relation to asthma and sex in children and adults.

Methods: Total IgE levels, skin prick test (SPT) positivity, eosinophil counts, and FEV₁ were assessed in 299 asthmatic cases (children and adults) recruited in chest clinics and 309 nonasthmatic population-based control subjects in the French Epidemiological Study on the Genetics and Environment of Asthma, Bronchial Hyperresponsiveness, and Atopy.

Results: Allergy parameters were significantly higher in asthmatic cases than in control subjects for children and adults and for both sexes. Sex and age modified the pattern of concordance of high IgE levels, SPT positivity, and eosinophilia among asthmatic cases, with the greatest overlap in male children (64%) and the lowest in female adults (24%). Patterns of change over the lifespan of IgE levels, eosinophil counts, and FEV₁/height² varied, with the acceleration of FEV₁ decrease being particularly evident in asthmatic adults. In adult cases and control subjects, SPT positivity (particularly to indoor allergens) was significantly related to IgE levels but not to eosinophil counts. The association of eosinophil counts with IgE levels was evident only in children. Environmental factors

(smoking, pets, and country living) did not alter the patterns observed.

Conclusions: Each allergy-related phenotype showed a distinct relation with asthma, with the role for eosinophils being different than that for IgE levels and SPT responses.

Clinical implications: Taking age and sex into account is essential for understanding the interrelationships of the various allergy-related phenotypes to asthma status. (J Allergy Clin Immunol ■■■■;■■■:■■■-■■■.)

Key words: Allergy, asthma, eosinophils, epidemiology, sex, IgE, lung function

The current debate regarding eosinophils no longer considers whether they are friend or foe¹ but rather focuses on determining the circumstances in which they act as a foe.^{2,3} Eosinophils were among the first allergy markers studied as potential risk factors for FEV₁ decrease in the context of the Dutch hypothesis,⁴ which postulates that features of asthma are risk factors of chronic obstructive pulmonary disease. Although investigations of skin prick test (SPT) responses and IgE levels support the hypothesis, the relation of various allergy traits to FEV₁ decrease varied between studies, suggesting disease heterogeneity.⁵⁻⁷ Skin test positivity, high serum total IgE levels, and peripheral blood eosinophilia likely represent different expressions of the atopic phenotype,^{8,9} which might explain their varied relation with FEV₁. Eosinophils and other allergy markers are strongly related to asthma, but not all asthmatic patients present with allergic traits, and the attributable risk of allergy in asthma has been questioned.¹⁰ Furthermore, the interpretation of the role of eosinophils is more nuanced after studies in adults have shown no effect of anti-IL-5 treatment, an eosinophil-specific cytokine,² on airways dysfunction in asthma.

Each marker of allergy shows a different relation with age, with eosinophil counts decreasing early in infancy, whereas IgE levels increase to early adulthood. Sex-related patterns have also been shown, with higher values of all parameters in male than in female subjects but stronger associations of eosinophils with asthma in women

From ^aINSERM, U780, Epidemiology and Biostatistics, Villejuif; ^bUniv Paris-Sud, IFR69, Villejuif; ^cINSERM, U794, Statistical Methods and Genetic Epidemiology of Multifactorial Diseases, Evry; ^dUniversité d'Evry, Evry; ^eUniversité René Descartes, Paris 5; ^fINSERM, U578, Grenoble; and ^gthe Arizona Respiratory Center, Tucson.

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The names of the members of the EGEA Cooperative Group are shown in the Appendix.

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Reprint requests: Marie-Pierre Oryszczyn, BSc, INSERM U780, 16 Avenue Paul Vaillant Couturier, 94807 Villejuif Cedex, France. E-mail: picot@vjf.inserm.fr.

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Abbreviations used

EGEA: Epidemiological Study on the Genetics and Environment of Asthma, Bronchial Hyperresponsiveness, and Atopy
 FEV_1/H^2 : $FEV_1/height^2$
 IU: International units
 SPT: Skin prick test
 SPTQ: Skin prick test quantitative score

with persistent asthma.¹¹ Different patterns of familial correlation of asthma-related phenotypes have been observed according to the mode of ascertainment of the families (offspring or parents).¹² In addition, environmental¹³⁻¹⁵ determinants of eosinophil counts, IgE levels, and skin test responses have been identified.

Few epidemiologic studies have collected data on the various markers simultaneously in subjects over the lifespan, and no study has assessed their interrelationships at the phenotypic level in relation to age, sex, and asthma status. The aim of the present study is to assess the relationships between total IgE levels, SPT responses, eosinophil counts, and FEV_1 in child and adult asthmatic cases and control subjects from the Epidemiological Study on the Genetics and Environment of Asthma, Bronchial Hyperresponsiveness, and Atopy (EGEA), taking into account asthma and sex. Specific consideration is given to eosinophils and to quantitative assessment of SPT responses, as recently validated.¹⁶

METHODS

Population

EGEA is a case-control and family study of adult and childhood asthma. Cases were recruited in chest clinics, as were population-based control subjects. The protocol has been described elsewhere.^{17,18} All subjects have been extensively characterized regarding phenotypic and environmental characteristics by using standardized questionnaires.^{13,15,17,18} The present analysis concerns 299 asthmatic cases and 309 nonasthmatic control subjects with data on IgE levels, SPT responses, eosinophil counts, and FEV_1 . The proportion of missing data (13.8%) was similar in cases and control subjects and by sex. Adult cases with missing data were older than other subjects (43.3 vs 37.8, $P = .03$). The study was approved by the appropriate institutional review board, and informed consent was obtained from all subjects (and from parents for the children; see additional Methods information in the Online Repository at www.jacionline.org).

Allergy markers, lung function, and asthma severity

Total IgE levels (in international units [IU] per milliliter) were measured by means of RIA.¹³ Specific IgE levels to a mixture of several inhalant allergens were assessed by using the Phadiatop (Pharmacia Diagnostics, AB, Guyancourt, France). The exact composition of the aeroallergens present in the Phadiatop has not been reported by the manufacturer, but information on its constituents is available. The percentage binding of the Phadiatop was classified as positive or negative according to the reference serum value. SPTs were performed to 11 allergens (including molds and indoor and

outdoor allergens). A quantitative score (the skin prick test quantitative score [SPTQ]) was constructed as the number of positive test results and validated regarding its biometric properties.¹⁶ Total and differential white blood cell counts were obtained by using standard methods, and the apparatuses used were those available in each center.

The maximum FEV_1 of 3 maneuvers was used to calculate the FEV_1 percent predicted value, correcting for height, age, and sex.

A score (1-4) was calculated to assess asthma severity, as previously described.¹⁹

Environmental factors

Smoking,¹³ contact with pets,²⁰ and country living (an indirect marker of contact with livestock),¹⁵ previously shown in the EGEA to relate to total IgE levels, SPT positivity (any wheal ≥ 3 mm), eosinophil counts, or FEV_1 , were considered in the adult analyses. Active smoking (nonsmokers, exsmokers, and smokers), childhood exposure to cat (≤ 16 years of age), and ever living in the country were included in models to assess whether the patterns observed between quantitative trait phenotypes were explained by environmental factors.

Statistical methods

Dichotomous variables used in analyses were SPT positivity, eosinophilia ($>5\%$), Phadiatop positivity, and high IgE levels (≥ 100 IU/mL). IgE levels and eosinophil counts were log transformed and expressed as geometric means. Analyses regarding eosinophil counts also used tertiles computed separately for adults and children. Standard statistical tests (linear and logistic regression) were used to assess the interrelations between asthma-associated traits in models, including age, sex, and environmental factors. Analyses on the relationships of eosinophils with FEV_1 were adjusted on age, sex, smoking, and inhaled steroid treatment and on age, sex, and smoking for other allergy parameters. SAS (version 9.1; SAS Institute, Inc, Cary, NC) software was used. (See additional Methods information in the Online Repository at www.jacionline.org).

RESULTS

Total IgE levels, Phadiatop positivity, SPT responses, and eosinophil counts considered either dichotomously or quantitatively were significantly higher for cases versus control subjects among both children and adults of both sexes (Table I). Both SPT positivity and high IgE levels were evident in about 90% of the child cases. The greatest difference between cases and control subjects in SPT responses was observed for indoor allergens, in particular for *Dermatophagoides pteronyssinus*, for which 75.6% versus 21.2% in pediatric cases and control subjects and 46.2% versus 15.3% in adult cases and control subjects had positive results. For outdoor allergens, timothy grass positivity was the most common (38.2% vs 18.2% in children and 27.3% vs 16.1% in adults, respectively). FEV_1 was significantly lower in cases than in control subjects, especially in adults.

Among cases, nearly all were in the active phase. More precisely, 111 (90.2%) children and 163 (92.6%) adults reported attacks in the last 12 months, and among those without attacks in that period, 5 children and 12 adults were using inhaled steroids. Among pediatric cases, 23%, 33%, 17%, and 27% were in each asthma severity category from mild to severe, with comparable proportions being 7%, 30%, 21%, and 42% in adults.

TABLE I. Characteristics of the population

	Female subjects				Male subjects			
	Children		Adults		Children		Adults	
	Cases (n = 39)	Control subjects (n = 45)	Cases (n = 87)	Control subjects (n = 111)	Cases (n = 84)	Control subjects (n = 41)	Cases (n = 89)	Control subjects (n = 112)
Age (y; mean $\pm$ SD)	11.1 $\pm$ 2.0	12.1 $\pm$ 2.2*	35.4 $\pm$ 13.3	40.0 $\pm$ 12.0*	11.3 $\pm$ 2.1	12.2 $\pm$ 2.3*	40.1 $\pm$ 15.0	43.6 $\pm$ 13.2†
Total IgE (IU/mL)								
GM (95% CI)	312 (36-2688)	36 (1-937)‡	161 (7-3874)	28 (1-542)‡	454 (49-4198)	68 (4-1551)‡	228 (20-2620)	36 (2-647)‡
IgE $\geq$ 100 IU/mL (%)	87.2	24.4‡	62.1	19.8‡	94.0	34.1‡	80.9	20.5‡
Specific IgE, Phadiatop positivity (%)	94.9	22.2‡	66.7	19.8‡	92.9	36.6‡	78.7	22.3‡
SPTs								
SPT positivity, any (%)	84.6	42.2‡	67.8	27.0‡	94.0	46.3‡	77.5	34.8‡
SPT positivity, indoor (%)	79.5	24.4‡	60.9	21.6‡	88.1	24.4‡	61.8	20.5‡
SPT positivity, outdoor (%)	51.3	18.2§	36.8	17.1	42.9	26.8†	46.1	18.8‡
SPT positivity, molds (%)	18.4	22.2	20.7	7.2§	23.8	17.5	24.7	10.7§
SPTQ, mean $\pm$ SD	1.9 $\pm$ 1.2	0.7 $\pm$ 0.9‡	1.7 $\pm$ 1.8	0.6 $\pm$ 1.3‡	2.2 $\pm$ 1.3	0.9 $\pm$ 1.2‡	2.0 $\pm$ 1.9	0.7 $\pm$ 1.1‡
Eosinophils (no./mm ³)								
GM (95% CI)	376 (94-1509)	168 (48-588)‡	260 (50-1342)	102 (19-557)‡	474 (136-1652)	212 (56-804)‡	207 (39-1096)	124 (32-475)‡
Eosinophils $>$ 5% (%)	56.4	13.3‡	40.2	7.2‡	67.9	22.0‡	24.7	7.1
FEV ₁ % predicted, mean $\pm$ SD	90.5 $\pm$ 9.8	95.4 $\pm$ 12.1*	89.9 $\pm$ 19.9	105.0 $\pm$ 13.1‡	92.1 $\pm$ 13.1	99.2 $\pm$ 10.4‡	85.5 $\pm$ 21.9	103.7 $\pm$ 15.0‡
FEV ₁ % predicted $<$ 80% (%)	15.4	8.9	27.6	0.9‡	16.7	2.4*	36.0	3.6‡

Comparison of cases and control subjects: * $P \leq .05$; † $P \leq .10$; ‡ $P \leq .001$; § $P \leq 0.01$.

GM, Geometric mean.

Sex and age

Among asthmatic cases, there was a greater overlap of the 3 allergy markers among children than among adults for both sexes (Fig 1). A substantial proportion (18.4%) of women with asthma had no marker of allergy, whereas 2.3% of male asthmatic cases lacked any marker of allergy ($P = .001$). Stratifying the sample according to smoking, country living, or pet exposure did not change the pattern.

The pattern of change with age of all quantitative parameters was studied cross-sectionally on the basis of asthma status. FEV₁/height² (FEV₁/H²) was used to adjust for the dimensional aspect of growth. Quadratic models gave the best fit for eosinophil counts and lung function with age, capturing the marked decrease of eosinophil counts during childhood and the pattern of lung growth during childhood followed by decrease in lung function in adulthood (Fig 2). The best fit for log IgE values was a linear decrease over the age range studied. For SPTQ scores, a nonsignificant decrease was observed after 35 years of age in both cases and control subjects. For all parameters, curves for allergic markers were higher but roughly parallel along the lifespan between cases and control subjects. For FEV₁/H², the curve of cases started at less than that of control subjects, remained parallel until midadulthood, and then showed an acceleration of

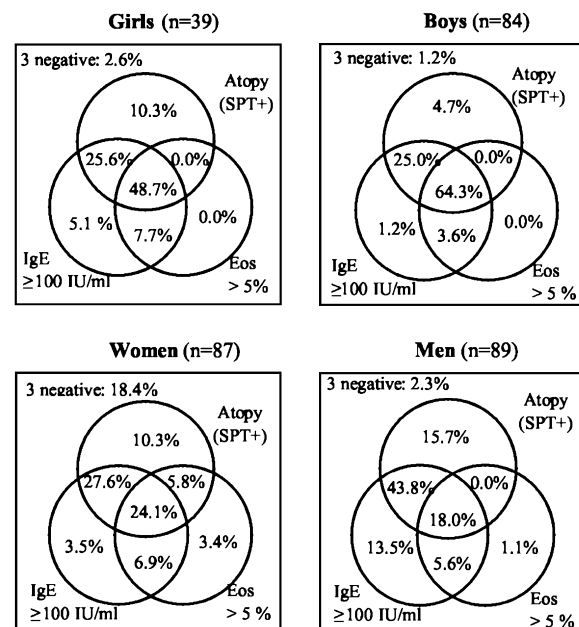


FIG 1. Concordance of SPT positivity, high IgE levels, and eosinophilia in asthmatic cases according to age and sex (Venn diagrams).

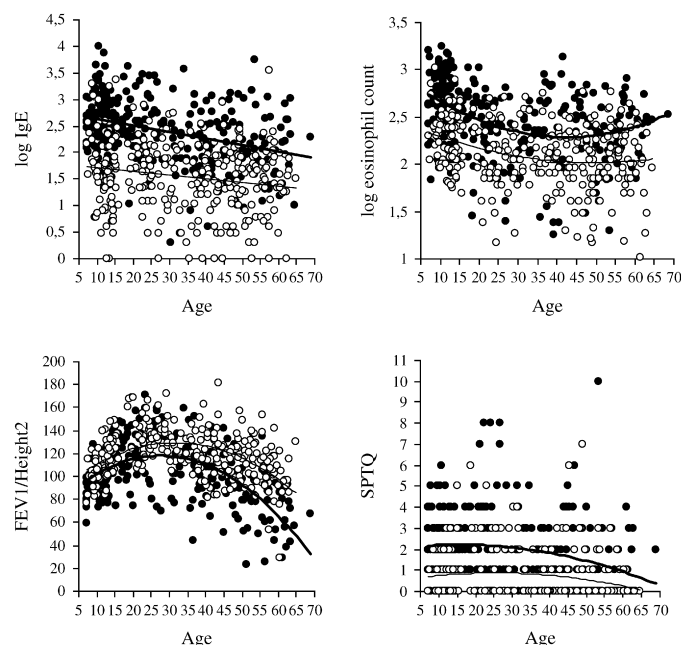


FIG 2. Associations of total IgE levels, eosinophil counts, and FEV_1/H^2 by age in cases and control subjects over the lifespan. Control subjects ($n = 309$) and patients ($n = 299$) are represented by open circles/thin curves and solid circles/thick curves, respectively. Cases and control subjects differed significantly for all parameters ($P < .001$). FEV_1/H^2 decrease with age among adults was significantly steeper in cases than in control subjects ($P < .001$).

decrease with age (Fig 2). A similar pattern was observed when considering FEV_1 expressed in liters or FEV_1 percent predicted (according to external reference values) instead of FEV_1/H^2 (data not shown). Separate models for male and female subjects were represented (see Fig E1 in the Online Repository at www.jacionline.org).

Among asthmatic cases, analyses taking the age of onset into account showed that adults with an early age of onset (<4 years) had higher SPTQ scores than those with an age of onset of 4 years or more and were younger at the time of the study. SPTQ scores were 1.96 versus 1.57 ($P = .002$) in women and 2.67 versus 2.03 (not significant) in men, respectively. The associations were similar after adjustment for age at assessment (or for age and smoking).

Relationships between total IgE levels, specific IgE levels, and SPT responses

Total IgE levels were significantly associated with SPT positivity in adult and child control subjects (73 versus 22 and 86 versus 31 IU/mL, respectively) and in adult cases (248 versus 96 IU/mL; all $P < .004$). In asthmatic children nearly all of whom had positive SPT responses, the association of IgE levels to SPT positivity and SPTQ scores did not reach statistical significance (see Table E1 in the Online Repository at www.jacionline.org), even after adjustment for age and sex. Among child control subjects, the association between SPT responses and IgE levels was strongest for outdoor allergens and remained significant after adjustment for age and sex. Among adult

cases and control subjects, the association between SPT responses and IgE levels were strongest for indoor allergens. Associations between total IgE levels and SPT positivity were not modified by adjustment for potential confounders (age, sex, smoking, and country living; see Table E1 in the Online Repository at www.jacionline.org).

The concordance of Phadiatop with SPT positivity was high, with overall κ coefficients being 0.49 and 0.44 for cases and control subjects among children and 0.68 and 0.54 for cases and control subjects among adults, respectively. Excluding *Aspergillus* and *Blattella* species from the definition of SPT positivity led to a slight increase in the κ statistic. Considering all subjects together, the positive and negative predictive values of Phadiatop for any of the 11 allergens were 90% and 78%, respectively.

Relationships of FEV_1 and asthma severity with IgE levels and SPT responses

In children FEV_1 was unrelated to IgE levels, SPT positivity, or SPTQ scores in cases and control subjects. In adult cases a significant positive association of FEV_1 percent predicted with SPT positivity was observed. However, nonatopic adult cases were 10 years older than atopic cases (46.9 versus 34.4 years), and the association of SPT positivity with FEV_1 disappeared after adjustment for age (84.2% vs 89.0%). No association was observed for FEV_1 with SPTQ score. Among asthmatic cases, associations of allergy markers with asthma severity scores showed that there was no clear pattern of IgE in relation to asthma

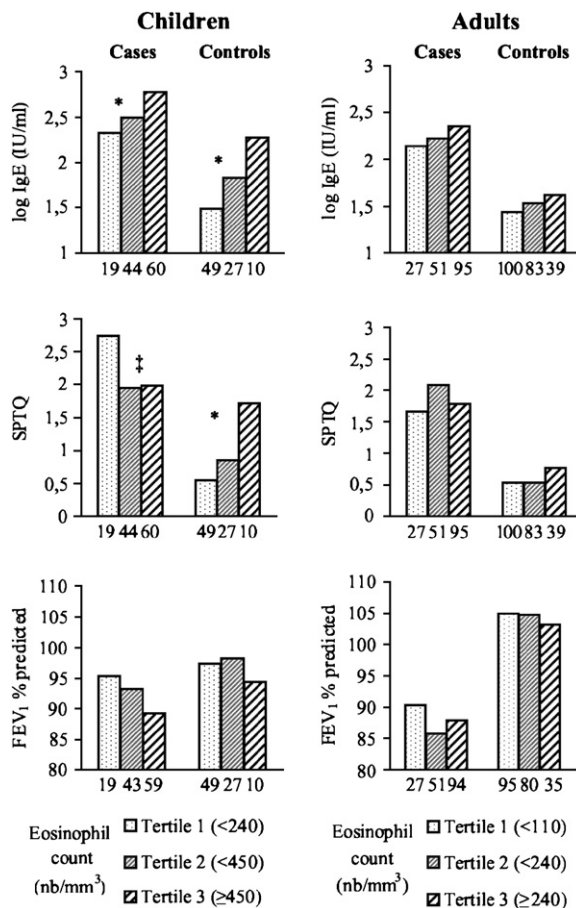


FIG 3. Associations of total IgE levels, SPTQ scores, and FEV₁ with eosinophil tertiles according to asthma in children and adults. Numbers of subjects in each group are shown below the bars. * $P \leq .01$; † $P \leq .05$.

severity, even after taking into account age, sex, and smoking in the analyses (see Table E2 in the Online Repository at www.jacionline.org).

Relationships between eosinophil counts with other allergy markers, FEV₁, and asthma severity

Because of the marked association of eosinophil counts with age, analyses of the relation with other allergy markers were conducted by using age-specific (children and adults) eosinophil tertiles (Fig 3). In children IgE levels increased significantly with eosinophil tertiles both in cases and in control subjects. For SPTQ scores, the pattern was complex: eosinophils were significantly inversely related to SPTQ scores in cases, whereas a significant positive association among control subjects was observed. Child cases in the lowest eosinophil tertile were more often receiving inhaled steroids than in the other tertiles (78.9%, 53.5%, and 55.9%, respectively). Eosinophils were unrelated to atopy, except among child control subjects, for whom the prevalence of atopy increased by eosinophil tertile (36.7%, 44.4%, and

80.0% of SPT positivity for the first, second, and third tertiles, respectively). For FEV₁, there was a nonsignificant inverse relation with eosinophils for both cases and control subjects; in adults there was no clear pattern. None of the associations of eosinophil tertiles with the other parameters were modified by taking environmental factors into account. Among asthmatic cases, no particular pattern was evidenced regarding the association of asthma severity scores and eosinophils (see Table E2 in the Online Repository at www.jacionline.org). Results were similar when inhaled steroid treatment was excluded from construction of the severity variable (data not shown).

DISCUSSION

The EGEA, with its well-characterized asthmatic cases and population-based control subjects, provides an opportunity to assess the interrelationships of various quantitative asthma-related phenotypes. In this study conducted both in children and adults, the interrelationships of asthma-related quantitative phenotypes depend not only on asthma but also on age and sex. These variables modified the pattern of association between IgE levels, SPT positivity, and eosinophilia among asthmatic cases, with the greatest overlap occurring in male children and the least in female adults. Eosinophils were significantly related to IgE levels and SPTQ scores in children only. Importantly, environmental factors, deleterious or protective, previously shown to relate to allergy markers did not explain the patterns observed.

We previously reported that eosinophilia was more strongly related to childhood-onset asthma among female subjects.¹¹ Here we show that sex had a marked influence not only on the level of allergy markers but also on their interrelationships. Among asthmatic cases, significantly more women than men did not have high IgE levels, atopy, and eosinophilia; this group has been little studied. Immunologic mechanisms specific to female subjects might be relevant, and preliminary results in the EGEA have shown a higher level of IgG₄ specific to cat both in female cases and control subjects.²¹ This finding suggests that sensitization, tolerance, and other mechanisms might depend on sex, possibly being hormone dependent. We previously showed that asthma severity increases with body mass index in women with early menarche only,²² but early menarche did not modify the present findings. These results add to the evidence that both the phenotypic presentation of asthma (here allergy) and the risk factors for asthma differ for female and male subjects,^{23,24} particularly in adulthood.

At any age, prevalence of high IgE levels, SPT responses, SPTQ scores, and eosinophilia was greater for cases than control subjects, indicating that these markers represent asthma-related quantitative phenotypes. However, it cannot be inferred from these associations that these markers are indeed true intermediate phenotypes (ie, on the causal pathway) as opposed to being associated traits.¹⁰ Nevertheless, the strength of the association is

a major argument to search genetic factors of these parameters, along with those of asthma. Indeed, genetic studies have increasingly focused on the quantitative traits associated with asthma because relations with these more specific phenotypes are likely to help disentangle the genetic determinants of this complex disease.

In our population the increase of IgE levels from early childhood to adulthood was not evident, as in Barbee et al,²⁵ likely because of the small number of control subjects in this age range and the bias introduced by recruiting of asthmatic cases through chest clinics. Whereas SPT responses, considered either dichotomously or quantitatively, were strongly related to IgE levels, eosinophil counts had markedly weaker associations with the other allergy parameters. Responsiveness at the skin level by using SPTs and an index of polysensitization (SPTQ and Phadiatop), showed that because of very strong association, Phadiatop does not bring additional information not already captured in SPTs. Furthermore, it must be emphasized that positivity to this test is not specific for single sensitization. Among children, IgE levels were most strongly associated with SPT responses to outdoor allergens, whereas among adults, the indoor allergens showed the strongest relation with IgE levels. Interestingly, adults with an early age of onset had higher SPTQ scores, confirming that not only childhood onset but also early-life onset is an interesting subphenotype of asthma to be considered in further studies. The lack of association of polysensitization with asthma severity in adults confirms previous observations from the EGEA.²⁶

In children only eosinophil counts were significantly related to IgE levels. Although we observed the expected relationship between atopy and eosinophil counts among child control subjects, it was not the case in asthmatic children because this relation appeared to be masked by medication use in the asthmatic children. The strength of the association of IgE levels with SPT responses was difficult to assess because nearly all asthmatic children had positive SPT responses. These findings add to the epidemiologic evidence that eosinophilia in childhood, which has been little studied, might represent a different inflammatory state than it does in adulthood and might be etiologically linked to the development of asthma in childhood. This observation is of potential clinical importance because studies questioning the efficacy of anti-IL-5 treatment have been conducted in adults only and thus cannot be directly extrapolated from adults to children.²⁷ Clearly more research is needed to understand the role of these cells in asthma.^{2,8,28-30} The lack of association of eosinophils with asthma severity observed here likely pertains to the observational nature of the population and the recruitment of asthmatic cases in chest clinics and thus does not conflict with the effect of inhaled steroids on eosinophils observed in clinical trials.³¹ The difference in FEV₁ between cases and control subjects, although apparent in childhood, was more noticeable in adulthood, which supports the speculation that remodeling-related events are dependent on the duration of asthma.³² In children parameters other than lung function

characterize asthma severity,^{33,34} and it would be of interest to assess whether the level of eosinophilia in asthmatic children predicts the persistence of severity of asthma into adulthood. Future epidemiologic studies should be undertaken to determine genetic and environmental determinants of eosinophilic inflammation and its role in allergy and asthma in each sex over the lifespan.

A major limitation of our analysis is the cross-sectional nature of the data, but no longitudinal study could easily provide data from 7 to 70 years. Because cohort effects likely modify the patterns, both cross-sectional and longitudinal studies are necessary to comprehensively approach lifespan patterns. A follow-up study currently in progress will help to assess whether factors related to low FEV₁ cross-sectionally, such as atopy, influence FEV₁ decrease over 10 years. A second limitation of our study was the difficulty of analyzing the pattern in control subjects, who appear more atopic than expected but who rarely present with eosinophilia. Although the recruitment of cases from chest clinics provided probands with a definitive diagnosis,¹⁷ it precludes the generalization of the findings to asthma in general. Studies in larger populations would be useful to assess the relationships between the various markers in nonasthmatic subjects.

Environmental factors undoubtedly play a key role in the increase of asthma incidence in the last decades, and substantial research is being directed to understanding the role of environmental factors on allergy-related phenotypes.^{13-15,21,35-37} However, environmental factors associated specifically with eosinophilia are not yet known. None of the environmental factors previously associated with allergy markers^{16,20} in the EGEA (active and passive smoking, country living, and early exposure to cat) modified, even slightly, the pattern of relationships between allergy markers or between allergy markers and FEV₁. This is an important unexpected result that suggests that genetic factors and, more likely, gene-environment interactions might play a major role in the phenotypic expression of asthma-related traits.

Overall, these results support the hypothesis that genetic factors control subphenotypes according to sex and age and that failure to address that complexity will hamper the unraveling of the cause of asthma and related phenotypes. At the clinical and biologic level, phenotypic heterogeneity might be explained by gene-environment interactions according to windows of exposure.³⁸⁻⁴⁰ In any case these results demonstrate that careful characterization of subphenotypes in allergy and asthma might improve our understanding of the cause of asthma and ultimately assist in the management of this complex disease.

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APPENDIX. EGEA COOPERATIVE GROUP

Coordination: F. Kauffmann; F. Demenais (genetics); I. Pin (clinical aspects).

Respiratory epidemiology: *INSERM U578, Grenoble:* V. Siroux; *INSERM U700, Paris:* M. Korobaef (EGEA1), F. Neukirch (EGEA1); *INSERM U707, Paris:* I. Annesi-Maesano; *INSERM U780, Villejuif:* F. Kauffmann, N. Le Moual, R. Nadif, M. P. Oryszczyn.

Genetics: *INSERM U393, Paris:* J. Feingold; *INSERM U535, Villejuif:* M. H. Dizier; *INSERM U794, Evry:* E. Bouzigon, F. Demenais; *CNG, Evry:* I. Gut, M. Lathrop.

Clinical centers: *Grenoble:* I. Pin, C. Pison; *Lyon:* D. Ecochard (EGEA1), F. Gormand, Y. Pacheco; *Marseille:* D. Charpin (EGEA1), D. Vervloet; *Montpellier:* J. Bousquet; *Paris-Cochin:* A. Lockhart (EGEA1), R. Matran (now in Lille); *Paris-Necker:* E. Paty, P. Scheinmann; *Paris-Trousseau:* A. Grimfeld, J. Just.

Data and quality management: *INSERM ex-U155 (EGEA1):* J. Hochez; *INSERM U780, Villejuif:* N. Le Moual, C. Ravault; *INSERM U794:* N. Chateigner; *Grenoble:* J. Ferran.

METHODS

Population

Cases were recruited in chest clinics in 5 cities through a standardized protocol, as were population-based control subjects. Inclusion criteria were place of birth, area of residence, age, familial structure, and a positive answer to 4 standardized questions based on international questionnaires: Have you ever had attacks of breathlessness at rest with wheezing? Have you ever had asthma attacks? Was this diagnosis confirmed by a physician? Have you ever had an asthma attack in the last 12 months? Of the population of 764 cases and control subjects from the EGEA, 53 asthmatic control subjects (16 women, 22 men, 4 girls, and 11 boys) and 6 subjects missing information on asthma were excluded.

Allergy markers, lung function, and asthma severity

Total IgE levels were measured by means of RIA (Phadebas PRIST technique; Pharmacia Diagnostics, AB, Guyancourt, France). Specific IgE levels to a mixture of several inhalant allergens (pollens, house dust mites, molds, and animal epithelia) were assessed by using the Phadiatop (Pharmacia Diagnostics, AB, Guyancourt, France). The exact composition of the aeroallergens present in the Phadiatop has not been reported by the manufacturer, but information on its constituents is available, and *Aspergillus* and *Blattella* species were not included in Phadiatop. SPT allergens studied were cat, *Dermatophagoides pteronyssinus*, *Cladosporium herbarum*, *Alternaria tenuis*, timothy grass, olive, birch, *Parietaria judaica*, ragweed, *Aspergillus* species, and *Blattella germanica*. The prevalence of positive results is expressed by using a cutoff of mean wheal diameter of 3 mm or greater than that elicited by the negative control.

Spirometry was conducted according to the European Community Respiratory Health Survey protocol, with slight modifications.^{E1} A Biomedin spirometer (Biomedin Srl, Padua, Italy) was used in all centers, except in Lyon, where a Pneumotach Jaeger spirometer (Jaeger) was used. The maximum FEV₁ of 3 maneuvers was used.

Predicted values for FEV₁ were derived from age, sex, and height on the basis of the 1983 European references^{E2} for subjects aged 18 years or more and based on those from Polgar and Weng^{E3} for those younger than 18 years.

A score (1-4) was calculated in 2 steps to assess asthma severity, as previously described.^{E4} In step 1 the sum of the following clinical items regarding the last 12 months was computed: frequency of attacks (<1 attack per month [0], <1 attack per week [1], <1 attack per day [2], and ≥1 attack per day [3]); symptoms between asthma attacks (none [0], wheezing [1], wheezing and dyspnea [2], and activities limited by dyspnea [3]); and hospitalization for asthma (no [0], yes [1]). Subjects were classified in 4 classes (0, 1, 2, and ≥3). Then in step 2 the 4-category variable built in step 1 was combined with treatment level (none, treatment without inhaled steroids, and treatment with inhaled steroids). This combination is derived from the last Global Initiative for Asthma guidelines,^{E5} here modified because information on inhaled steroid dose was unavailable.

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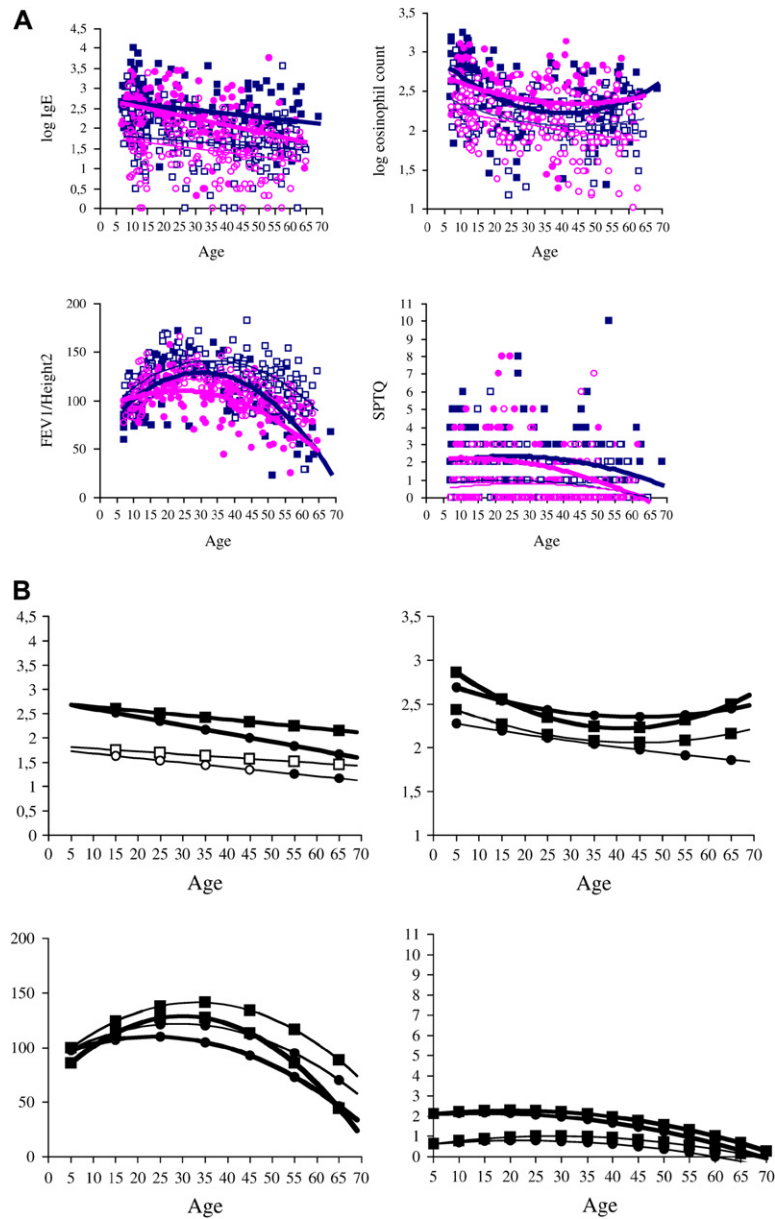


FIG E1. A, Associations of total IgE levels, eosinophil counts, and FEV₁/H² by age and sex in cases and control subjects over the lifespan. The best fit was obtained considering age for IgE levels and age² for eosinophil counts and FEV₁/H². Male and female control subjects are represented by *open squares* and *circles/thin curves* and male and female cases by *solid squares* and *circles/thick curves*. **B,** Associations of total IgE levels, eosinophil counts, and FEV₁/H² by age and sex in cases and control subjects over the lifespan (tendency curves only). The best fit was obtained considering age for IgE levels and age² for eosinophil counts and FEV₁/H². Male and female control subjects are represented by *open squares* and *circles/thin curves* and male and female cases by *solid squares* and *circles/thick curves*.

TABLE E1. IgE level (in international units per milliliter) according to SPT responses

	Children				Adults			
	Cases		Control subjects		Cases		Control subjects	
	Unadjusted	Adjusted*	Unadjusted	Adjusted*	Unadjusted	Adjusted†	Unadjusted	Adjusted†
SPT positivity, any allergen								
No	235	228	31	30	96	137	22	22
Yes	425‡	398	86§	86	248	260§	73	68
SPT positivity, indoor allergen								
No	348	386	36	37	100	127	25	25
Yes	413	324	118§	120§	288	308	82	80
SPT positivity, outdoor allergen								
No	380	358	32	32	146	189	25	25
Yes	433	397	189	218	282§	261	100	93

Indoor allergens include cat, *Dermatophagoides pteronyssinus*, and *Blattella germanica*. Outdoor allergens include timothy grass, olive, birch, and ragweed. IgE levels are expressed as geometric means.

Comparison of SPT negativity and SPT positivity: ‡ $P \leq .10$; || $P \leq 0.001$; § $P \leq .01$.

*Adjusted on age and sex.

†Adjusted on age, sex, smoking, and country living.

TABLE E2. Relationships between asthma severity and IgE levels, eosinophil counts, and SPTQ scores

Asthma severity score*	Children				Adults			
	n	IgE (IU/mL)	SPTQ score	Eosinophil counts (nb/mm ³)	n	IgE (IU/mL)	SPTQ score	Eosinophils (nb/mm ³)
1	25	319.5	2.12	437.2	11	207.0	1.82	210.2
2	36	433.0	2.11	401.0	47	194.2	2.15	231.2
3	19	415.9	2.11	509.5	32	167.3	1.53	239.2
4	30	404.9	1.93	445.4	66	236.7	1.91	226.9
<i>P</i> value		.78	.93	.66		.73	.56	.98

IgE levels and eosinophil counts are expressed as geometric means.