The asthma-rhinitis multimorbidity is associated with IgE polysensitization in adolescents and adults

Valérie Siroux [1], Natalia Ballardini [2,3,4], Marion Soler [1], Christian Lupinek [5], Anne Boudier [1], Isabelle Pin [1,6], Jocelyne Just [7,8], Rachel Nadif [9,10], Josep M Anto[11], Erik Melen [2,3], Rudolf Valenta [5], Magnus Wickman [2,3,12], Jean Bousquet [9,10]

[1] University Grenoble Alpes, Inserm, CNRS, IAB, 38000, Grenoble, France

[2] Institute of Environmental Medicine, Karolinska Institutet, Stockholm, Sweden

[3] Sachs' Children and Youth Hospital, Södersjukhuset, Stockholm, Sweden

[4] St John's Institute of Dermatology, King's College London, London, UK

[5] Division of Immunopathology, Department of Pathophysiology and Allergy Research, Center for Pathophysiology, Infectiology and Immunology, Medical University of Vienna, Austria

[6] CHU Grenoble Alpes, Department of Pediatrics, Grenoble, France

[7] Allergology department, Children Hospital Armand Trousseau, Paris, France

[8] Inserm, UMR-S 1136 INSERM and UPMC Paris, France

[9] Inserm, U1168, VIMA: Aging and chronic diseases. Epidemiological and public health approaches, F-94807, Villejuif, France

[10] Univ Versailles St-Quentin-en-Yvelines, UMR-S 1168, F-78180, Montigny le Bretonneux, France

[11] ISGLoBAL, Centre for Research in Environmental Epidemiology (CREAL), Barcelona, Spain

[12] Centre for Clinical Research Sörmland, Uppsala University, Eskilstuna Sweden

Short Title

Asthma-rhinitis multimorbidity and IgE polysensitization

Corresponding author

Valérie Siroux

Institut pour l'Avancée des Biosciences

Centre de Recherche UGA / Inserm U 1209 / CNRS UMR 5309

Equipe d'épidémiologie environnementale

Site Santé - Allée des Alpes

38700 La Tronche

Valerie.siroux@univ-grenoble-alpes.fr

Tel : +33 4 76 54 95 56

Manuscript word count: 3780

Abstract word count: 249

Abstract

Background: Children with multimorbid asthma and rhinitis show IgE polysensitization to several allergen sources. This association remain poorly studied in adolescents and adults using defined allergen molecules. We investigated IgE sensitization patterns towards a broad panel of aeroallergen components in adults and adolescents with a focus on individuals with asthma and rhinitis multimorbidity.

Methods: IgE reactivity to 64 microarrayed aeroallergen molecules was determined with the MeDALL-chip in samples from the French EGEA study (n=840, age=40.7±17.1) and the Swedish population-based birth cohort BAMSE (n=786, age=16±0.26). The age- and sex-adjusted associations between the number of IgE-reactive allergen molecules (≥0.3 ISU) and the asthma-rhinitis phenotypes were assessed using a negative binomial model.

Results: Groups representing four phenotypes were identified: no asthma-no rhinitis (A-R-; 30% in EGEA and 54% in BAMSE), asthma alone (A+R-; 11% and 8%), rhinitis alone (A-R+; 15% and 24%), and asthma-rhinitis (A+R+; 44% and 14%). The numbers of IgE-reactive aeroallergen molecules significantly differed between phenotypes (median in A-R-, A+R-, A-R+ and A+R+: 0, 1, 2 and 7 in EGEA and 0, 0, 3, and 5 in BAMSE). As compared to A-R- subjects, the adjusted ratio of the mean number of IgE-reactive molecules was higher in A+R+ than in A+R- or A-R+ (10.0, 5.4 and 5.0 in EGEA and 7.2, 0.7 and 4.8 in BAMSE).

Conclusion: The A+R+ phenotype combined the sensitization pattern of both the A-R+ and A+R-phenotypes. This multimorbid polysensitized phenotype seems to be generalizable to various ages and allergenic environments and may be associated with specific mechanisms.

Key words

Allergens, Epidemiology, Multimorbidity, Polysensitization, Specific-IgE

Abbreviations

A: Asthma

EGEA: Epidemiological study of the Genetics and Environment of Asthma, bronchial hyperresponsivness and atopy

FP7: Framework Programme 7 (European Union)

IgE: Immunoglobulin E

ISAC: Immuno solid-phase allergen chip

ISU: Standardized units for specific IgE

MeDALL: Mechanisms of the development of allergy

R: Rhinitis

Introduction

Allergic diseases are complex and represent multiple phenotypes (1) such as clinically silent IgE sensitization, isolated phenotypes such as only rhinitis, asthma, dermatitis, food allergy, and multimorbidities associating several conditions in the same subject.

In children, multimorbidities of allergic diseases share common causal mechanisms that are partly IgE-mediated.(2) IgE sensitization is heterogeneous and important clinical and immunological differences exist between mono- and poly- sensitized patients.(3-5) An important result from the FP7-funded project MeDALL (Mechanisms of the Development of Allergy) was the identification of the multimorbid polysensitized phenotype of allergic diseases in children, characterized by children with asthma presenting both allergic polysensitization and other allergic diseases, mainly rhinitis.(5) Although polysensitization and allergic-related multimorbidity were known, they had not been associated in a single phenotype. In MeDALL, using hypothesis and data-driven methods, this novel phenotype was shown to be associated with some important features of allergic diseases including a low probability of remission of IgE sensitization and symptoms,(6-8) elevated levels of total and specific IgE,(9) high levels of blood eosinophils, and a high rate of allergy in family history. This newly described phenotype is also associated with the severity of asthma,(10-13) rhinitis,(11-13) atopic dermatitis(14) or food allergy.(15) However, this phenotype needs to be confirmed also in adolescents and adults, to understand whether these associations are transient or persistent along the life course. In a previous analysis in adults from the Epidemiological study of the Genetics and Environment of Asthma, bronchial hyperresponsiveness and atopy - EGEA study, first evidence was provided that polysensitization is associated with multimorbid asthma and rhinitis.(16) However, the analysis was limited by use of allergen extracts which

represent mixtures of source-specific genuine allergen molecules and cross-reactive allergens so that a true discrimination of co- and cross-sensitization could not be made (17, 18) and by the fact that only a limited number of allergen extracts could be tested by skin prick testing.

Until recently, the IgE sensitization profiling in allergic multimorbidity has been based on a limited number of allergens. Currently, the allergen micro-array technology makes it possible to measure allergen-specific IgE antibody responses to a large number of allergen molecules. In MeDALL, the immunoCAP ISAC chip technology (19, 20) was refined to increase its sensitivity and to incorporate new allergens.(20) The resulting MeDALL chip has been validated and tested in birth cohorts and allows measuring IgE responses to more than 170 allergen components with a sensitivity similar to that of the traditional ImmunoCAP as validated using the major birch pollen allergen Bet v 1-specific IgE.(21-23)

The present cross-sectional study investigates if the multimorbid polysensitized phenotype suspected in children could be generalized to adults and adolescents living in two different allergenic environments (France and Sweden) using different cohort designs (i.e., a cohort following a case-control study: EGEA, France (24) and a birth cohort: BAMSE, Sweden (25)). IgE sensitization patterns towards a large variety of respiratory allergen components were measured using the MeDALL micro-array technology.(21, 23)

Methods

Populations and study setting

The present analysis relies on two populations, the EGEA study and the BAMSE birth cohort.

The EGEA1 study is composed of a group of 388 individuals with asthma enrolled in chest

clinics, 1,244 first-degree relatives and 415 control individuals.(26)Participants were recruited through self-completed questionnaires and had a complete examination including pulmonary function tests.(24) About 11 years later, 1,601 participants (77.1% of the original cohort + 58 new family members) took part in a complete examination (EGEA2). The analysis of IgE was conducted with serum samples from 840 adults at EGEA2 (463 with and 377 without asthma). Written consent was obtained from all participants. Ethical approval was obtained from the ethics committees (Necker-Enfants Malades Hospital, Paris for EGEA2).

BAMSE is a population-based birth cohort comprising 4,089 children from representative areas of Stockholm.(25) For this study, questionnaire data from baseline (2 months), 1, 2, 4, 8, 12 and 16 years were used. Blood was drawn at 4, 8 and 16 years. Sera were available for 64%, 60%, and 62% of the population.(6, 7) In 1,699 children, 42% of the original cohort, blood samples were available from all three clinical follow-ups (4, 8 and 16 years). Of these children, a subset of 800 was randomly selected. Permission for the study was obtained from the Regional Ethical Review board at Karolinska Institutet at each follow up and parents of participating children gave their informed consent.

Definition of asthma, allergic rhinitis and atopic dermatitis

Validated questionnaires were used in both studies. In EGEA, ever asthma was defined by a positive answer to either ‘‘*Have you ever had attacks of breathlessness at rest with wheezing?*’’ or ‘‘*Have you ever had asthma attacks*?’’ or being recruited as an asthma case. Allergic rhinitis ever was defined by a positive answer to “*Have you ever had allergic rhinitis?*” or “*Have you ever had hay fever?*”. The age of asthma onset was estimated by questionnaire.(27) Age of rhinitis onset could not be evaluated since the questionnaire did not

include this information. Atopic dermatitis was defined by a positive response to "*Have you ever had atopic dermatitis?*".

In BAMSE asthma at age 1 and 2 years was defined as at least three episodes of wheeze in combination with inhaled corticosteroids and/or signs of bronchial hyperreactivity without concurrent upper respiratory infection the past year before follow-up.(9) Asthma at 4, 8, 12 and 16 years was defined as more than 3 episodes of wheeze in the last 12 months prior to the date of questionnaire and/or at least 1 episode of wheeze in the last 12 months in combination with inhaled steroids occasionally or regularly. Allergic rhinitis was defined as symptoms of sneezing, a runny or blocked nose, or itchy, red and watery eyes after exposure to furred pets or pollen in the past 12 months and/or doctor's diagnosis of allergic rhinitis since last follow up. Participants that fulfilled rhinitis definition or asthma definition at least one during the follow-ups at 1, 2, 4, 8, 12 or 16 years were classified as rhinitis ever and asthma ever. Atopic dermatitis was defined as dry skin, itchy rashes with age-specific location for 2 weeks or more in the past 12 months and/or doctor's diagnosis of atopic dermatitis.

Participants from EGEA and BAMSE were grouped in four phenotypes: no asthma & no rhinitis (A-R-), asthma alone (A+R-), rhinitis alone (A-R+), and asthma & rhinitis (A+R+). Multimorbidity was defined as having both asthma and rhinitis. For an exploratory analysis, we further considered atopic dermatitis.

Allergen-specific IgE measurement

IgE reactivity to microarrayed allergens was determined in anonymized samples with the MeDALL-chip (multiplex microarray) in EGEA2 samples and in the 16-years old samples in BAMSE.(21, 23, 28, 29) The MeDALL-chip comprises 176 allergen components including aero-

and food allergen components. In the present study IgE responses to 64 defined clinically relevant respiratory allergenic molecules were studied (Table S1 in the *Supplements*). The measurement of allergen-specific IgE was performed as described.(6, 21) Results are given in ISU. According to previous studies, the positivity of each allergen-specific IgE was defined with a threshold of 0.3 ISU IgE.(6, 21, 22). As previously shown, the agreement between SPT and allergen-specific IgE in EGEA was strong (Cohen Kappa coefficient ≥ 0.65), with slightly higher prevalences using the MeDALL-chip (except for ragweed) (Table S2 in the *Supplements*).(28) In BAMSE, agreement for allergen-specific IgE between Immunocap and the MeDALL-chip components was high (Cohen Kappa coefficient ≥ 0.75) for the most prevalent allergens (thimothy grass, birch and cat) and moderate for the less prevalent allergens (house dust mite, horse and mugwort), as expected given the link between the kappa and the prevalence (Table S3 in the *Supplements*).

Within each study, respiratory allergenic molecules recognized by ≥ 3% of subjects were considered to address the sensitization profiles of asthma and rhinitis phenotypes (Table S1).

Biases

In both studies, allergen-specific IgE was measured in a subset of samples (for cost issues), randomly selected among the BAMSE samples and in EGEA among the non-asthma and asthma samples separately. Within each group, the selection process was unrelated to the allergen exposure and the allergen-specific IgE reactivity, minimizing the risk for selection bias (Fig. S1 and S2).

Statistical methods

Demographic results are given in means ± SD or n (%). For the analysis of the number of positive allergen-specific IgE, results are given in medians and 25-75 percentiles (Q1 and Q3). The level of allergen-specific IgE for each positive allergen molecule were compared between phenotypes using the non-parametric Kruskall Wallis test. Negative binomial regression model was applied to estimate age and sex adjusted associations with the number of positive allergen-specific IgE. This regression models the ratio of the mean number of positive allergen-specific IgE (and its 95% confidence interval) between the asthma and rhinitis phenotypes. For example, a ratio of 5.0 for asthma means that those with asthma had a 5-fold higher mean number of positive allergen-specific IgE, as compared with those without asthma. Logistic regression models were used to estimate Odds Ratio (OR) and their 95% CI associated with positive allergen-specific IgE.

Results

Demographic characteristics of participants

There were 840 adult participants to the French EGEA study (463 with and 377 without asthma) and 786 adolescents of the Swedish BAMSE cohort (Fig. S1 and S2). In BAMSE and within the asthma and the non-asthma group in EGEA, participants included in the analysis did not differ to those non-included regarding allergy related characteristics (Tables S4 and S5). In the BAMSE study, included participants had more often asthma than non-included ones (22.9% *vs.* 18.0%, p=0.02). The demographic characteristics of the participants are presented in Table 1. In EGEA, mean age of asthma onset was higher in the A+R- than in the A+R+ (19.4 (± 16.0) *vs.* 13.7 (± 14.6), p=0.002). The frequency of asthma-rhinitis multimorbidity (A+R+)

was 44% in EGEA and 14% in BAMSE. Asthma only (A+R-) and rhinitis only (A-R+) were found in 11% and 15% in EGEA and 8% and 24% in BAMSE.

IgE reactivity to allergen molecules

We identified 39 and 18 allergens recognized by IgE in at least 3% of the EGEA population and the BAMSE population, respectively (Table S1). IgE-reactivities to olive Ole e 1 and house dust mite Der p 1 were frequent in EGEA (20.4% and 26.4% respectively) and infrequent in BAMSE (1.2% and 1.3%). IgE-reactivity to birch Bet v 1 was frequent in BAMSE. IgE-reactivities to animal (cat, dog and horse) and grass pollen allergens were similar in both populations irrespective of age difference between the two studies.

Multimorbidity and polysensitization

The number of subjects with IgE reactivity to at least one allergen and the number of IgE-reactive allergens differed significantly between the asthma-rhinitis phenotypes in both studies. IgE sensitizations to few allergens were observed in the A-R- phenotype whereas the greatest number of IgE-reactive allergens was found in the multimorbid A+R+ phenotype (Table 2, Fig. 1-A). The A+R- and A-R+ phenotypes showed IgE reactivity to significantly fewer allergens than the A+R+ phenotype ($p<0.001$). As compared to A-R- subjects, the age and sex adjusted ratio of the mean number of IgE sensitizations was significantly higher in A+R+ than in A+R- or A-R+, in both cohorts (Table 2). There was a difference between EGEA and BAMSE since the A+R- group showed IgE reactivity to significantly more allergens in EGEA than in BAMSE.

In EGEA, the number of allergen molecules associated with specific IgE was significantly higher in childhood-onset asthma than in adult-onset asthma (median were 9.0 and 2.0, respectively, $p < 0.0001$) (Fig. 1B and Table 2). In both childhood and adult-onset asthma, the A+R+ phenotype exhibited IgE reactivity to a higher number of allergens than the A+R- ($p<0.001$ in childhood-onset asthma and $p=0.002$ in adult-onset asthma) (Table 2). Among subjects with rhinitis, the number of allergen recognized by IgE was higher among subjects with childhood-onset asthma as compared to adult-onset asthma ($p<0.0001$).

In both studies, the prevalence of subjects with IgE reactivity to at least one allergen and the number of IgE-reactive allergens increased gradually with the number of diseases (asthma and rhinitis and atopic dermatitis) (Table 3). For each additional allergic disease phenotype, the number of IgE-reactive allergens increased significantly. However, among subjects with asthma and rhinitis, atopic dermatitis was not associated with the number of IgE-reactive allergens (age and sex adjusted relative change [95% CI] = 1.1 [0.9-1.3], $p=0.29$ in EGEA and 1.2 [0.8-1.7], $p=0.41$ in BAMSE). The median level of allergen-specific IgE among positive allergen molecule increased with the number of diseases ($p<0.0001$ in EGEA and BAMSE, Table 3), although in EGEA the trend was not linear, with a median level of similar magnitude in participants with 2 and 3 diseases.

Sensitization patterns between asthma and rhinitis phenotypes

The patterns of IgE sensitizations differed between the phenotypes and populations (Fig. 2 and 3). In the A-R- phenotype, IgE-reactivity frequency was below 10% for all allergen components in both cohorts, except for Phl p 1 in BAMSE (Fig. 2-A, 3-A). In the A+R- phenotype, Phl p 1, most of the Der p allergens and Fel d 1 were over 10% in EGEA (Fig. 2-B),

whereas only Phl p 1 was over the threshold of 10% in BAMSE (Fig. 3-B). In the A-R+ phenotype, pollen allergens and Fel d 1 were over 10% in both EGEA and BAMSE (Fig. 2-C and 3-C). In BAMSE Can f 5 was also over the threshold of 10% recognition in the A-R+ phenotype. In the A+R+ phenotype (Fig. 2-D and 3-D), 24 allergen molecules in EGEA (61.5% of the 39 allergens recognised by at least 3% of the EGEA population) and 17 allergens in BAMSE (94.4% of the 18 allergens recognised by at least 3% of the BAMSE population) were over the 10% IgE recognition frequency. Although the A+R- and A-R+ sensitization patterns differed between the EGEA and BAMSE populations, in both studies the A+R+ sensitization pattern results from the combination of the A+R- and A-R+ sensitization patterns.

For the three allergens with the highest IgE-reactivity frequency in the A-R- (Phl p 1, Ole e 1, and Der p 23 in EGEA with IgE-reactivity frequency in A-R- of 9.5%, 6.0% and 7.0%; Phl p 1, Bet v 1 and Fel d 1 in BAMSE with IgE-reactivity frequency in A-R- of 15.6%, 7.6% and 5.9%, respectively) we estimated the risk for A-R+, A+R- and A+R+ as compared to A-R- (Fig. 4). In both studies and for each allergen component, the OR was always the highest for A+R+, although the risk in A+R+ did not statistically differ from the risk in A-R+ or A+R-. In EGEA, the OR point estimate for A+R+ was approximately the addition of the OR point estimate for A+R- and A-R+. In BAMSE, none of these three allergen molecules was associated with the asthma only phenotype (OR point estimates were close to 1).

Discussion

Our study shows that the asthma-rhinitis multimorbid phenotype is associated with strong IgE-polysensitisation, both in adolescents and in adults. The asthma-rhinitis multimorbid

phenotype combined the sensitization pattern of both the asthma-only phenotype and the rhinitis-only phenotype. In addition, the systematic highest risk observed for allergen specific-IgE reactivity in the asthma-rhinitis multimorbid phenotype as compared to the asthma- or rhinitis-only phenotypes, indicates that the mechanisms involved in the asthma-rhinitis multimorbid phenotype might be partly different or enhanced than those involved in asthma- or rhinitis-only phenotypes. The observation found in two different populations with exposure to different allergen sources, the Swedish BAMSE cohort and the French EGEA population, provides evidence for the generalizability of this "extreme" phenotype to any age or allergenic environment.

A major strength of our study is the strategy used, by addressing the research question in two populations differing regarding many features including population age (including adolescents and adults), study design (birth cohort and case-control combined to a family study) and allergen exposure due to different environments (Sweden with a high birch pollen exposure and France with a high mite exposure). Long-term birth cohort studies are essential to understand disease mechanisms and life course of allergic diseases,(30) but in population-based studies individuals with severe disease are rare. Therefore, patient cohorts can be combined with population-based studies to fill gaps of unmet needs of birth cohorts (30) for a better definition of disease phenotypes and stratification. Similar observations in the two populations provide evidence that the observed associations are not due to uncontrolled bias affecting a single study or by chance. A further asset relies in the precise characterisation of the allergic sensitization, by assessing IgE sensitization to well-defined allergen molecules which allow to discriminate co- and cross-sensitization. The MeDALL micro-array has been established and was carefully validated regarding specificity and sensitivity in the FP7-funded EU program MeDALL and has previously been successfully used in several studies including

the EGEA and BAMSE cohorts.(7, 22, 28)

One limitation, relying on the cross-sectional design of our analyses, is the lack of definite information on the timing of the events and of the successive specific allergens sensitization. This timing of events has been addressed in some birth cohort studies. However, none of these previous studies has used defined allergen molecules but only allergen extracts to detect IgE sensitization. A recent study in the MAS and PASTURE cohorts, which identified different sensitisation profiles characterized by allergen specificity, time course and sIgE level, did not identify clear temporal sensitization pattern between 1 and 6 years of age.(31) The clusters were mainly characterized by allergen specificity and strength of the sIgE sensitization. In the Paris cohort, Gabet et al identified as early as 18 months of age three sensitization profiles, including a "polysensitized" profile which exhibits the highest risk for multimorbidity at 6 years.(8) In the EGEA study, we examined the impact of age of asthma onset on the sensitization patterns. When the disease started in childhood, the number of sensitizations was greater than when the disease started later for both A+R- and A+R+ groups. But, the association between the asthma-rhinitis multimorbidity and allergic polysensitization was observed in both childhood- and adult-onset phenotypes. Another limitation could be that IgE measurements on the chip are performed under conditions of low amounts of allergen immobilized to the solid phase which may be affected by allergen-specific IgG antibodies. However, the MeDALL allergen-chip has been carefully evaluated with respect to sensitivity and was found to be more sensitive for detecting IgE sensitization than allergen-extract-based skin prick testing and conventional allergen extract-based serology because significantly more sensitized subjects were detected.(22)

Our study is clinically relevant and provides results with a general validity because it examines asthma-rhinitis multimorbidity and polysensitization in depth and confirms the hypothesis raised in the recent paper by Burte et al in the same EGEA cohort using classical IgE tests for 12 aeroallergens sources.(16) It is novel as it uses the most advanced method of IgE measurement that is needed to accurately assess true polysensitization, allowing for the assessment of IgE-rectivity to define allergen molecules. Moreover, similar findings were observed in a second cohort which allow the generalizability of the results. For the first time, our study showed that for specific allergens with high IgE recognition frequency in the study populations, the risk of IgE-reactivity was the highest in the asthma-rhinitis multimorbid phenotype as compared to the isolated asthma or rhinitis phenotypes. This might suggest that the biological mechanisms involved in the association between IgE-sensitization and the AR phenotypes are partly different or enhanced in the AR multimorbidity phenotype. Moreover, in the EGEA study we observed that the magnitude of the risk for the AR multimorbidity phenotype was roughly the addition of the risk observed in asthma alone and rhinitis alone phenotypes. This might suggest independent effects of allergen-specific IgE-sensitisation in asthma and in rhinitis (if the effects were mainly shared, the risk in A+R+ would not be higher than the risk in A+R- or A-R+). Our observations provide hypotheses for novel biological explanations which warrants further investigations.

The major difference across the two study populations is related to the allergen exposure. In Sweden, exposure to mites is very low, and low level of IgE-reactivity to mite allergens probably explains differences in the total number of allergen recognized between EGEA and BAMSE and the lack of sensitization in A+R- patients in Sweden. Moreover, in Sweden birch pollen is highly prevalent whereas it is not common in most parts of France.(7, 28) Thus, the prevalence of birch-related IgE is higher in Sweden than in France. Finally, Ole e 1 is the major

allergen of olive and it displays strong cross-reactivity with Fra e 1, a major ash allergen.(32, 33) These two species are common in France but not in Sweden, which explain the low prevalence of Ole e 1-specific IgE in BAMSE as compared to EGEA.

If we consider the impact of environmental exposure, the results are similar in both studies and show that allergic multimorbidity is associated with IgE-polysensitization in adults (France) and adolescents (Sweden). Moreover, the levels of allergen-specific IgE are associated with the number of co-existing allergic disease phenotypes. Our study extends to adolescents and adults recent findings in childhood studies and therefore indicates that the allergy multimorbid IgE-polysensitized phenotype starts early in life and does not remit over time, but seems to remain persistent across the life course. In addition, we observed that AR multimorbidity is associated with IgE sensitization to significantly more allergen molecules as compared with asthma alone both in childhood- and adult-onset asthma, emphasizing the generalizability of this phenotype. Recent studies in children showed that this phenotype is associated with the severity of asthma and rhinitis,(11-13, 15) and therefore underline the relevance of this phenotype at the clinical and public health level. By investigating pathways related to asthma severity in children with asthma and rhinitis, Liu et al. showed that allergy was associated with asthma severity through several pathways, from allergic inflammation and subsequently through pulmonary physiology or rhinitis severity.(12)

Our study clearly demonstrated different patterns of sensitization according to the asthma and rhinitis phenotypes, with no or few sensitizations in the A-R- group, with no or predominant sensitization to indoor allergens in A+R-, predominant pollen allergens in A-R+, and frequent IgE-sensitization to both pollen and indoor allergens in A+R+. Differences in the A+R- sensitization patterns between EGEA and BAMSE might partly be explained by

differences in allergenic environment, in particular the infrequent sensitization to house dust mite allergens in Sweden, and differences in the asthma phenotypes considered (e.g. EGEA also includes adult-onset asthma, which might have different IgE-sensitization pattern).

In conclusion, our study provides new insights into the patterns of allergic sensitization across the AR phenotypes in both adolescents and adults. By showing that the allergy multimorbid polysensitized phenotype, previously identified in early life, seems to remain persistent across the life-course, our findings advocate for paying a particular attention to this specific phenotype, to identify its underlying mechanisms and risk factors.

Acknowledgments

The authors thank all those who participated to the setting of the EGEA and BAMSE studies and on the examinations of the individuals. The authors are grateful to the three CIC-Inserm of Necker, Grenoble and Marseille who supported the EGEA study and in which participants were examined. They are indebted to all the individuals who participated to the EGEA study without whom the study would not have been possible. In addition, the authors thank the children and parents participating in the BAMSE cohort.

We thank the Epidemiological Study on Genetics and Environment of Asthma (EGEA) cooperative group members as follows. **Coordination**: V Siroux (epidemiology, PI since 2013); F Demenais (genetics); I Pin (clinical aspects); R Nadif (biology); F Kauffmann (PI 1992-2012). **Respiratory epidemiology**: Inserm ex-U 700, Paris: M Korobaeff (Egea1), F Neukirch (Egea1); Inserm ex-U 707, Paris: I Annesi-Maesano (Egea1-2); Inserm ex-U 1018, Villejuif: F Kauffmann, MP Oryszczyn (Egea1-2); Inserm U 1168, Villejuif: N Le Moual, R Nadif, R Varraso; Inserm U 1209 Grenoble: V Siroux. **Genetics**: Inserm ex-U 393, Paris: J Feingold; Inserm U 946, Paris: E Bouzigon, F Demenais, MH Dizier; CNG, Evry: I Gut (now CNAG, Barcelona, Spain), M Lathrop (now Univ McGill, Montreal, Canada). **Clinical centers**: Grenoble: I Pin, C Pison; Lyon: D Ecochard (Egea1), F Gormand, Y Pacheco; Marseille: D Charpin (Egea1), D Vervloet (Egea1-2); Montpellier: J Bousquet; Paris Cochin: A Lockhart (Egea1), R Matran (now in Lille); Paris Necker: E Paty (Egea1-2), P Scheinmann (Egea1-2); Paris-Trousseau: A Grimfeld (Egea1-2), J Just. **Data and quality management**: Inserm ex-U155 (Egea1): J Hochez; Inserm U 1168, Villejuif: N Le Moual; Inserm ex-U780: C Ravault (Egea1-2); Inserm ex-U794: N Chateigner (Egea1-2); Grenoble: J Quentin (Egea1-2).

Conflict of interests statements

Rudolf Valenta has received research grants from Biomay AG, Vienna, Austria and Virvaxx, Vienna, Austria. He serves as a consultant for Biomay AG, Vienna, Austria, Viravaxx, Vienna, Austria and Fresenius Medical Care, Bad Homburg, Germany. Valerie Siroux has received speaker honorarium from TEVA, AstraZeneca and Novartis-France, outside the submitted work. Jean Bousquet serves in scientific advisory boards from Almirall, Meda, Merck, MSD, Novartis, Sanofi-Aventis, Takeda, Teva, Uriach. Magnus Wickman has received research grants from ThermoFisher Scientific. Jocelyne Just serves in scientific advisory boards from - ALK, Stallergenes Greer, Thermofisher, AstraZeneca and Novartis-France, outside the submitted work.

Authors contributions

V Siroux, C Lupinek, I Pin, J Just, R Nadif, JM Anto, E Melen, R Valenta, M Wickman and J Bousqet contributed to the data acquisition. V Siroux, N Balardini, M Soler and A Boudier conducted the statistical analyses. V Siroux and J Bousquet drafted the first version of the manuscript. All authors contributed to the interpretation of the data, critically revised the manuscript and approved the final version.

Sources of funding: The study was supported in part by Inserm Aviesan Itmo santé publique, the Scientific committee “AGIR for chronic diseases”, ANR-PRSP 2009, grant F4605 of the Austrian Science Fund (FWF) to RV and by the European Commission’s Seventh Framework Program MeDALL under grant agreement no. 261357. In addition, the BAMSE study was

427 supported by grants from the Swedish Heart-Lung Foundation, the Swedish Research Council
428 and Stockholm County Council.

References

1. Zellweger F, Eggel A. IgE-associated allergic disorders: recent advances in etiology, diagnosis, and treatment. *Allergy* 2016;**71**:1652-1661.

2. Pinart M, Benet M, Annesi-Maesano I, von Berg A, Berdel D, Carlsen KC, et al. Comorbidity of eczema, rhinitis, and asthma in IgE-sensitised and non-IgE-sensitised children in MeDALL: a population-based cohort study. *Lancet Respir Med* 2014;**2**:131-140.

3. Custovic A, Sonntag HJ, Buchan IE, Belgrave D, Simpson A, Prosperi MC. Evolution pathways of IgE responses to grass and mite allergens throughout childhood. *J Allergy Clin Immunol* 2015;**136**:1645-1652 e1641-1648.

4. Bousquet J, Anto JM, Bachert C, Bousquet PJ, Colombo P, Crameri R, et al. Factors responsible for differences between asymptomatic subjects and patients presenting an IgE sensitization to allergens. A GALEN project. *Allergy* 2006;**61**:671-680.

5. Bousquet J, Anto JM, Wickman M, Keil T, Valenta R, Haahtela T, et al. Are allergic multimorbidities and IgE polysensitization associated with the persistence or re-occurrence of foetal type 2 signalling? The MeDALL hypothesis. *Allergy* 2015;**70**:1062-1078.

6. Westman M, Lupinek C, Bousquet J, Andersson N, Pahr S, Baar A, et al. Early childhood IgE reactivity to pathogenesis-related class 10 proteins predicts allergic rhinitis in adolescence. *J Allergy Clin Immunol* 2015;**135**:1199-1206 e1191-1111.

7. Asarnoj A, Hamsten C, Waden K, Lupinek C, Andersson N, Kull I, et al. Sensitization to cat and dog allergen molecules in childhood and prediction of symptoms of cat and dog allergy in adolescence: A BAMSE/MeDALL study. *J Allergy Clin Immunol* 2016;**137**:813-821 e817.

8. Gabet S, Just J, Couderc R, Bousquet J, Seta N, Momas I. Early polysensitization is associated with allergic multimorbidity in PARIS birth cohort infants. *Pediatr Allergy Immunol* 2016;**27**:831-837.

9. Ballardini N, Bergstrom A, Wahlgren CF, van Hage M, Hallner E, Kull I, et al. IgE antibodies in relation to prevalence and multimorbidity of eczema, asthma, and rhinitis from birth to adolescence. *Allergy* 2016;**71**:342-349.

10. Just J, Saint-Pierre P, Gouvis-Echraghi R, Laoudi Y, Roufai L, Momas I, et al. Childhood allergic asthma is not a single phenotype. *J Pediatr* 2014;**164**:815-820.

11. Zoratti EM, Krouse RZ, Babineau DC, Pongracic JA, O'Connor GT, Wood RA, et al. Asthma phenotypes in inner-city children. *J Allergy Clin Immunol* 2016;**138**:1016-1029.

12. Liu AH, Babineau DC, Krouse RZ, Zoratti EM, Pongracic JA, O'Connor GT, et al. Pathways through which asthma risk factors contribute to asthma severity in inner-city children. *J Allergy Clin Immunol* 2016;**138**:1042-1050.

13. Pongracic JA, Krouse RZ, Babineau DC, Zoratti EM, Cohen RT, Wood RA, et al. Distinguishing characteristics of difficult-to-control asthma in inner-city children and adolescents. *J Allergy Clin Immunol* 2016;**138**:1030-1041.

14. Amat F, Saint-Pierre P, Bourrat E, Nemni A, Couderc R, Boutmy-Deslandes E, et al. Early-onset atopic dermatitis in children: which are the phenotypes at risk of asthma? Results from the ORCA cohort. *PLoS One* 2015;**10**:e0131369.

15. Just J, Elegbede CF, Deschildre A, Bousquet J, Moneret-Vautrin DA, Crepet A, et al. Three peanut-allergic/sensitized phenotypes with gender difference. *Clin Exp Allergy* 2016;**46**:1596-1604.

16. Burte E, Bousquet J, Siroux V, Just J, Jacquemin B, Nadif R. The sensitization pattern differs according to rhinitis and asthma multimorbidity in adults: the EGEA study. *Clin Exp Allergy* 2017;**47**:520-529.

17. Kazemi-Shirazi L, Niederberger V, Linhart B, Lidholm J, Kraft D, Valenta R. Recombinant marker allergens: diagnostic gatekeepers for the treatment of allergy. *Int Arch Allergy Immunol* 2002;**127**:259-268.

18. Pfiffner P, Stadler BM, Rasi C, Scala E, Mari A. Cross-reactions vs co-sensitization evaluated by in silico motifs and in vitro IgE microarray testing. *Allergy* 2012;**67**:210-216.

19. Hiller R, Laffer S, Harwanegg C, Huber M, Schmidt WM, Twardosz A, et al. Microarrayed allergen molecules: diagnostic gatekeepers for allergy treatment. *FASEB J* 2002;**16**:414-416.

20. Canonica GW, Ansotegui IJ, Pawankar R, Schmid-Grendelmeier P, van Hage M, Baena-Cagnani CE, et al. A WAO - ARIA - GA(2)LEN consensus document on molecular-based allergy diagnostics. *World Allergy Organ J* 2013;**6**:17.

21. Lupinek C, Wollmann E, Baar A, Banerjee S, Breiteneder H, Broecker BM, et al. Advances in allergen-microarray technology for diagnosis and monitoring of allergy: the MeDALL allergen-chip. *Methods* 2014;**66**:106-119.

22. Skrindo I, Lupinek C, Valenta R, Hovland V, Pahr S, Baar A, et al. The use of the MeDALL-chip to assess IgE sensitization: a new diagnostic tool for allergic disease? *Pediatr Allergy Immunol* 2015;**26**:239-246.

23. Bousquet J, Anto J, Auffray C, Akdis M, Cambon-Thomsen A, Keil T, et al. MeDALL (Mechanisms of the Development of ALLergy): an integrated approach from phenotypes to systems medicine. *Allergy* 2011;**66**:596-604.

24. Kauffmann F, Dizier MH, Annesi-Maesano I, Bousquet J, Charpin D, Demenais F, et al. EGEA (Epidemiological study on the Genetics and Environment of Asthma, bronchial hyperresponsiveness and atopy)-- descriptive characteristics. *Clin Exp Allergy* 1999;**29 Suppl 4**:17-21.

25. Wickman M, Kull I, Pershagen G, Nordvall SL. The BAMSE project: presentation of a prospective longitudinal birth cohort study. *Pediatr Allergy Immunol* 2002;**13 Suppl 15**:11-13.

26. Kauffmann F, Dizier MH, Pin I, Paty E, Gormand F, Vervloet D, et al. Epidemiological study of the genetics and environment of asthma, bronchial hyperresponsiveness, and atopy: phenotype issues. *Am J Respir Crit Care Med* 1997;**156**:S123-129.

27. Bouzigon E, Corda E, Aschard H, Dizier MH, Boland A, Bousquet J, et al. Effect of 17q21 variants and smoking exposure in early-onset asthma. *N Engl J Med* 2008;**359**:1985-1994.

28. Siroux V, Lupinek C, Resch Y, Curin M, Just J, Keil T, et al. Specific IgE and IgG measured by the MeDALL allergen-chip depend on allergen and route of exposure: The EGEA study. *J Allergy Clin Immunol* 2017;**139**:643-654.

29. Wickman M, Lupinek C, Andersson N, Belgrave D, Asarnoj A, Benet M, et al. Detection of IgE Reactivity to a Handful of Allergen Molecules in Early Childhood Predicts Respiratory Allergy in Adolescence. *EBioMedicine* 2017.

30. Bousquet J, Gern JE, Martinez FD, Anto JM, Johnson CC, Holt PG, et al. Birth cohorts in asthma and allergic diseases: report of a NIAID/NHLBI/MeDALL joint workshop. *J Allergy Clin Immunol* 2014;**133**:1535-1546.

31. Hose AJ, Depner M, Illi S, Lau S, Keil T, Wahn U, et al. Latent class analysis reveals clinically relevant atopy phenotypes in 2 birth cohorts. *J Allergy Clin Immunol* 2017;**139**:1935-1945.

32. Imhof K, Probst E, Seifert B, Regenass S, Schmid-Grendelmeier P. Ash pollen allergy: reliable detection of sensitization on the basis of IgE to Ole e 1. *Allergo J Int* 2014;**23**:78-83.

33. Barderas R, Purohit A, Rodriguez R, Pauli G, Villalba M. Isolation of the main allergen Fra e 1 from ash (Fraxinus excelsior) pollen: comparison of the natural and recombinant forms. *Ann Allergy Asthma Immunol* 2006;**96**:557-563.

Figures legend

FIGURE 1. Distribution of the number of IgE-reactive allergens (≥0.3 ISU) according to the asthma and rhinitis phenotypes in EGEA and in BAMSE (A) and taking into account age of asthma onset in EGEA (B) (among the 64 allergen components).

In these box plots, the bottom and the top of the rectangle indicate the first and the third quartile, respectively, and the horizontal line near the middle of the rectangle indicates the median. The vertical lines from the top and the bottom of the rectangle indicate the maximum and minimum values, respectively. The diamond indicates the mean value.

FIGURE 2. IgE recognition frequencies and intensities to respiratory allergens recognized by >3% of the EGEA samples according to the combined asthma and rhinitis phenotypes in EGEA with: (A) no asthma and no rhinitis (A-R-), (B) asthma but no rhinitis (A+R-), (C) no asthma but rhinitis (A-R+), and (D) asthma and rhinitis (A+R+)

FIGURE 3. IgE recognition frequencies and intensities of respiratory allergens recognized >3% of the BAMSE samples according to the combined asthma and rhinitis phenotypes in BAMSE with: (A) no asthma and no rhinitis (A-R-), (B) asthma but no rhinitis (A+R-), (C) no asthma but rhinitis (A-R+), and (D) asthma and rhinitis (A+R+)

FIGURE 4. Age- and sex-adjusted association (OR (95% CI)) between the asthma-rhinitis phenotypes and the three allergen components with the highest IgE-reactivity frequency in the A-R- group. A) In EGEA, B) in BAMSE