COBRAPed cohort: Sensitization patterns do not differentiate children with severe asthma from those with a milder disease

Stephanie Lejeune¹, Naïm Bouazza², Pascale Nicaise³, Valérie Jolaine², Lea Roditis⁴, Christophe Marguet⁵, Flore Amat⁶, Patrick Berger⁷, Michael Fayon⁸, Jean-Christophe Dubus⁹, Sophie Valois¹⁰, Philippe Reix¹¹, Mathieu Pellan¹², Brouard Jacques¹³, Raphaël Chiron¹⁴, Lisa Giovannini-Chami¹⁵, Jacques de Blic², A. Deschildre¹, and Guillaume LEZMI²

¹Centre d’Infection et d’Immunité de Lille
²Hôpital universitaire Necker-Enfants malades
³Hôpital Bichat - Claude-Bernard
⁴Centre Hospitalier Universitaire de Toulouse
⁵Centre Hospitalier Universitaire de Rouen
⁶Hôpital Universitaire Mère-enfant Robert-Debre
⁷INSERM U1045
⁸Centre Hospitalier Universitaire de Bordeaux
⁹Assistance Publique Hôpitaux de Marseille
¹⁰Centre Hospitalier Universitaire Grenoble Alpes Hôpital Couple Enfant
¹¹Hospices Civils de Lyon
¹²Hôpital Jean Verdier
¹³Centre Hospitalier Universitaire de Caen
¹⁴Centre Hospitalier Regional Universitaire de Montpellier
¹⁵Hôpitaux Pediatriques de Nice CHU-LENVAL

September 5, 2023

Abstract

Background: It is unclear whether sensitization patterns differentiate children with severe recurrent wheeze (SRW) / severe asthma (SA) from those with non-severe recurrent wheeze (NSRW) / non-severe asthma (NSA). Our objective was to compare the sensitization patterns between children with SRW/SA and NSRW/NSA from the French COBRAPed cohort. Methods: IgE to 112 components (c-sIgE) (ImmunoCAP® ISAC) were analyzed in 125 preschool (3-6 years) and 170 school-age children (7-12 years). Supervised analyses and clustering methods were applied to identify patterns of sensitization among children with positive c-sIgE. Results: We observed c-sIgE sensitization in 51% of preschool and 75% of school-age children. Sensitization to house dust mite (HDM) components was more frequent among NSRW than SRW (53% vs 24%, p<0.01). Sensitization to non-specific lipid transfer protein (nsLTP) components was more frequent among SA than NSA (16% vs 4%, p<0.01) and associated with a FEV1/FVC <-1.64 z-score. Among sensitized children, seven clusters with varying patterns were identified. The two broader clusters identified in each age group were characterized by “few sensitizations, mainly to HDM”. One cluster (n=4) with “multiple sensitizations, mainly to grass pollen, HDM, PR-10, and nsLTP” was associated with SA in school-age children. Conclusions: Although children with wheeze/asthma display frequent occurrences and high levels of sensitization, the sensitization patterns did not clearly discriminate children with severe disease from those with milder disease. These results suggest that the severity of wheeze/asthma may depend on both IgE- and non-IgE-mediated mechanisms.
Title:

COBRAPed cohort: Sensitization patterns do not differentiate children with severe asthma from those with a milder disease

Short Title: Allergen sensitization in the COBRAPed cohort

Stéphanie Lejeune, MD,PhD1,2, Naïm Bouazza, PhD3, Pascale Roland Nicaise, PharmD, PhD4,5, Valérie Jolaine3, Léa Roditis, MD6, Christophe Marguet, MD, PhD7, Flore Amat, MD, PhD8, Patrick Berger, MD, PhD9, Michael Fayon, MD, PhD10, Jean-Christophe Dubus, MD, PhD11, Sophie Valois, MD12, Philippe Reix, MD, PhD13, Mathieu Pellan, MD14, Jacques Brouard, MD, PhD15, Raphael Chiron, MD16, Lisa Giovannini-Chami, MD, PhD17, Jacques de Blic, MD, PhD18, Antoine Deschildre, MD14, and Guillaume Lezmi, MD, PhD18 on behalf of the COBRAPed Study Group.

1Univ. Lille, CNRS, Inserm, CHU Lille, Institut Pasteur de Lille, U1019 - UMR 9017 - CIIL - Center for Infection and Immunity of Lille, Pediatric Pulmonology and Allergy Department. Hôpital Jeanne de Flandre, F-59000 Lille, France
2Univ. Lille, LIRIC UMR 995 Inserm, Clinical Investigation Center, CIC-1403-Inserm-CHU, Lille, France
3Unité de Recherche Clinique-Centre Investigation Clinique, APHP, Hôpital Necker-Enfants malades, Paris, France
4Immunology Department, Hôpital Bichat, APHP, Paris, France
5Inserm, PHERE, Université Paris Cité, Paris, France
6Children Hospital, Pediatric Pulmonology and Allergy Unit CHU Toulouse, Toulouse, France
7EA3830-GHRV, Rouen University, France, Pediatric Respiratory and Allergic Diseases, CF Reference Center, Rouen University Hospital-Charles Nicolle, Rouen, France
8Robert Debré Hospital, Pediatric Pulmonology and Allergy, University of Paris Cité, INSERM UMR 1018, Paris, France
9Univ. Bordeaux, Centre de Recherche Cardio-Thoracique de Bordeaux, INSERM, U1045, Centre d’Investigation Clinique (CIC 1401), Bordeaux, France
10CHU de Bordeaux, Unité de Pneumologie Pédiatrique, Centre d’Investigation Clinique (CIC 1401), Bordeaux, France
11Unité de Pneumopédieatrie CHU Timone-Enfants, Aix-Marseille Université, IRD MEPHI, IHU Méditerranée-Infection, Marseille, France
12Pédiatrie. CHU Grenoble Alpes; INSERM, Institute for Advanced Biosciences; Université Grenoble Alpes; Grenoble, France
13Service de Pneumologie, Allergologie et Mucoviscidose Pédiatrique, CHU de Lyon, Lyon, France. UMR 5558 (EMET), CNRS, LBBE, Université de Lyon, Villeurbanne, France
14Service de Pédiatrie, CHU Jean Verdier, Bondy, France
15Service de Pédiatrie Médicale, CHU Caen, Caen, France. Groupe de Recherche sur l’Adaptation Microbienne (GRAM 2.0), Normandie Université, Caen, France
16Pediatric Department, Montpellier University Hospital, Montpellier, France
17Pediatric Pulmonology Department, Lenval University Hospital, Nice, France
18Department of Pediatric Pulmonology and Allergy, AP-HP, Hôpital Necker-Enfants Malades, F-75015, Université Paris Cité, Paris, France
Members of the COBRAPed Study Group:

Dr Rola Abou-Taam (CHU Necker-Enfants Malades), Dr Muriel Le Bourgeois (CHU Necker-Enfants Malades), Dr Alice Hadchouel-Duvergé (CHU Necker-Enfants Malades), Dr David Drummond (CHU Necker-Enfants Malades), Pr Christophe Delacourt (CHU Necker-Enfants Malades), Dr Marie-Alexandra Alyanaki-an (CHU Necker-Enfants Malades), Pr Lucienne Chatennoud (CHU Necker-Enfants Malades), Dr Caroline Thumerelle (CHU Lille), Dr Clémence Mordacq (CHU Lille), Dr Irina Badiu-Decleyre (CHU Lille), Dr Cécile Bonnel (CHU Lille), Dr Laure Delbecque (CHU Lille), Dr Laurent Beghin, PhD (CHU Lille), Mrs Graziella Mingardi (CHU Lille), Mrs Caroline Tournegros (CHU Grenoble Alpes), Pr Sylvain Blanchon (CHU Lille), Dr Léa Roditis (CHU Toulouse), Pr Véronique Houdoin (Robert Debré Hospital, Paris), Dr Stéphanie Wannin (Robert Debré Hospital, Paris), Dr Marie Noelle Lebras (Robert Debré Hospital, Paris), Dr Stéphane Debelleix (CHU Bordeaux), Dr valérie Siao (CHU Bordeaux), Mrs Marine Servat (CHU Bordeaux, Hôpital Haut-Lévêque), Mr Guillaume Simon (CHU Bordeaux, Hôpital Pellegrin-Enfants), Dr Patricia El Boustany (CHU Marseille Timone-Enfants), Dr Emmanuelle Bosdure (CHU Marseille Timone-Enfants), Dr Julie Mazenq (CHU Marseille Timone-Enfants), Dr Isabelle Cabon (CHU Marseille Timone-Enfants), Dr Camille Ohlmann (CHU Lyon), Dr Stéphanie Vrielynck (CHU Lyon), Dr Virginie Jubin (CHU Lyon), Dr Sylvie-Anne André Gomez (CHU Lyon), Dr Marie-Christine Werck Gallois (CHU Lyon), Dr Priscille Biermé (CHU Lyon), Dr Isabelle Pin (CHU Grenoble) and Pr Sylvie Chollet-Martin (Immunology Department, Hôpital Bichat).

Acknowledgements

The authors would like to thank the patients and their families for their participation in the study, as well as all nurses, physicians, and clinical research coordinators who were involved in the COBRAPed study group.

Corresponding author:
Stéphanie Lejeune
stephanie.lejeune@chu-lille.fr

Key words: asthma, preschool, school-age, sensitization, severe asthma

Word count: 3,414

Abstract word count: 237

Number of figures: 2

Number of tables: 4

List of abbreviations

ACT: asthma control test
BD: bronchodilator
BMI: body mass index
CCD: cross-reactive carbohydrate determinants
c-sIgE: component-specific IgE
FEV1: forced expiratory volume in one second
FVC: forced vital capacity
HDM: house dust mite
ICS: inhaled corticosteroids
IgE: type E immunoglobulin
Abstract

**Background:** It is unclear whether sensitization patterns differentiate children with severe recurrent wheeze (SRW) / severe asthma (SA) from those with non-severe recurrent wheeze (NSRW) / non-severe asthma (NSA). Our objective was to compare the sensitization patterns between children with SRW/SA and NSRW/NSA from the French COBRAPed cohort.

**Methods:** IgE to 112 components (c-sIgE) (ImmunoCAP® ISAC) were analyzed in 125 preschool (3-6 years) and 170 school-age children (7-12 years). Supervised analyses and clustering methods were applied to identify patterns of sensitization among children with positive c-sIgE.

**Results:** We observed c-sIgE sensitization in 51% of preschool and 75% of school-age children. Sensitization to house dust mite (HDM) components was more frequent among NSRW than SRW (53% vs 24%, p<0.01). Sensitization to non-specific lipid transfer protein (nsLTP) components was more frequent among SA than NSA (16% vs 4%, p<0.01) and associated with a FEV1/FVC <-1.64 z-score. Among sensitized children, seven clusters with varying patterns were identified. The two broader clusters identified in each age group were characterized by “few sensitizations, mainly to HDM”. One cluster (n=4) with “multiple sensitizations, mainly to grass pollen, HDM, PR-10, and nsLTP” was associated with SA in school-age children.

**Conclusions:** Although children with wheeze/asthma display frequent occurrences and high levels of sensitization, the sensitization patterns did not clearly discriminate children with severe disease from those with milder disease. These results suggest that the severity of wheeze/asthma may depend on both IgE- and non-IgE-mediated mechanisms.

**Key Messages**

Children with wheeze/asthma display frequent occurrences and high levels of sensitization, but c-sIgE sensitization patterns do not clearly discriminate between non-severe and severe recurrent wheeze / asthma. Sensitization to non-specific lipid transfer protein (nsLTP) components was more frequent among SA than NSA, and was associated with lung function impairment. Cluster analysis of the results for sensitized children identified seven clusters, of which the two largest were characterized by “few sensitizations, mainly to house dust mite (HDM)”. Only one small cluster consisting of “multiple sensitizations, including to nsLTP”, was associated with severe asthma at school-age.

**Introduction**
Severe asthma (SA) in school-age children (7-12 years) and severe recurrent wheeze (SRW) in preschool children (3-6 years) affect less than 5% of children with asthma (1). They are heterogeneous conditions characterized by multiple phenotypes based on various features such as an association with other atopic conditions, environmental factors, lung function impairment, type of underpinning inflammation, or allergenic sensitization (2–4).

Several studies have highlighted the impact of sensitization in the natural history of asthma. In particular, early and multiple occurrences of sensitization have been shown to be associated with severe persistent asthma and lung function impairment throughout childhood (5–9). However, it is still unclear whether severity in preschool and school-age children is underpinned by different patterns of sensitization (10). Component resolved-diagnostics (CRD) detects IgE specific to individual allergen molecules (components, c-sIgE) rather than whole extracts and has been used in previous studies to characterize sensitization profiles in children (5–7,10). Previous results from the Pediatric Cohort of Bronchial Obstruction and Asthma (COBRAPed), a French multicenter prospective observational cohort of preschool (3-6 years) and school-age children (7-12 years) with recurrent wheeze/asthma, suggest a role for both environmental factors and atopy in asthma severity (11). Thus, the description of sensitization profiles using CRD in this well-described population provides an opportunity to further study the relationship between allergic sensitization and asthma severity during childhood. The main objective of our study was to determine whether sensitization patterns (biological sources and allergen components) can discriminate between children with NSRW/NSA and those with SRW/SA.

Methods

Study design and participants

COBRAPed is a prospective, observational, multicenter cohort study that was initiated in France, and has been described elsewhere (11). Institutional ethical approval (CPP Ile de France 1) and written informed consent from parents/caregivers of all participants were obtained. The study is registered in ClinicalTrial.gov (NCT02114034).

Preschool (3-6 years) and school-age (7-12 years) children were enrolled and assigned to one of four groups: (a) non-severe preschool recurrent wheezers (NSRW), (b) severe preschool recurrent wheezers (SRW), (c) non-severe school-age asthmatic children (NSA), and (d) severe school-age asthmatic children (SA). Preschool children with SRW and school-age children with SA had persistent symptoms or a need for short-acting β-agonist (SABA) and/or frequent or severe exacerbations and/or persistent airflow obstruction for school-age children, despite being treated with high doses of inhaled corticosteroids (ICS) and another controller therapy (12). A full description of the cohort, inclusion and exclusion criteria, and details of the clinical assessments has been previously published (11) and is available in the Online Supporting Information. Atopy was defined as having at least one positive skin-prick test (SPT) (wheal diameter ≥3 mm) and/or specific IgE levels (≥0.35 kuA/l) against common airborne and/or food allergens. Patients with SRW and SA receiving anti-IgE treatment with omalizumab were excluded for this analysis.

Detection and classification of component-specific IgE antibodies

IgE to 112 allergenic components were measured using an ImmunoCAP Immuno Solid-Phase Allergen Chip (ISAC) (Thermo Fisher/Phadia A, Uppsala, Sweden). Levels of component-specific IgE (c-sIgE) antibodies were reported in ISAC Standardized Units (ISU). Sensitization was defined at the c-sIgE level and biological source level. To determine sensitization at the c-sIgE level, depending on the nature of the analysis, we dichotomized c-sIgE using a binary threshold (< or ≥0.30 ISU) or based on the supplier’s four-group categorical classification (negative: <0.3 ISU, low: 0.3-1 ISU, medium/high: ≥1-15 ISU, very high: ≥15 ISU), depending on the nature of the analysis (Fig 1) (10,13,14). Sensitization was also defined at the biological source level based on the food/airborne biological sources (e.g., egg, cow’s milk, etc.) or molecular family for cross-reactive components (e.g., PR-10: pathogenesis-related protein family 10 (PR-10), etc.).

Statistical analysis
R version 3.3.1 statistical software was used for statistical analysis (R Core Team (2016) Vienna, Austria). Continuous variables are presented as medians [interquartile range] and categorical variables as numbers (%). Comparisons of quantitative data were performed using Wilcoxon-Mann-Whitney tests. Categorical variables were analyzed using the chi-square test or Fisher exact test as appropriate. The number of positive biological sources by age was evaluated using a quasi-Poisson regression to account for over-dispersion issues. No imputation of missing data was performed. Heatmaps were used to visualize the data using graphical representation as a grid of colors (according to the level of c-sIgE ISU), with rows standing for individuals and columns standing for components. The heatmaps were stratified according to severity group and individuals were ordered by age.

Both unsupervised and supervised analyses were performed to assess underlying data correlations. Components with a positive response (≥0.3 ISU) for at least three subjects and participants with at least one c-sIgE ≥0.3 ISU were retained for these analyses (Sup Fig.1). Principal component analyses (PCAs) were performed within the R function “prcomp”. Biplots of the principal components derived from the PCAs were plotted based on the classification of severe/non-severe disease. Then, random forest analyses using the known severity class of the patients were performed. Receiver operating characteristic (ROC) curves were used to assess the performance of the model using all c-sIgE to perform the classification and appraise the model predictions. The area under the curve (AUC) values indicated the level of precision: excellent for an AUC between 0.90 and 1.00; good for an AUC between 0.80 and 0.90; fair for an AUC between 0.70 and 0.80; poor for an AUC between 0.60 and 0.70, and fail for an AUC between 0.50 and 0.60. The prediction errors of the random forest analyses were also assessed by calculating the out-of-bag (OOB) errors. Furthermore, an unsupervised clustering approach was applied to identify patterns of c-sIgE sensitization among participants. Sensitization clusters were derived by clustering participants using Bayesian estimations of a mixture of Bernoulli distributions (Bernoulli Mixture Model), as previously described in detail (15). The BayesBinMix R package (15) was used to join estimation of the number of clusters and model parameters of the Bernoulli mixture model using Markov chain Monte Carlo sampling. A Poisson prior distribution was applied for the number of clusters and a uniform distribution for the Bernoulli parameters.

Results

Description of the population

Among the 329 children included in the COBRAPed cohort with available ISAC data, 6 SRW and 28 SA receiving omalizumab at inclusion were excluded. Among the 295 remaining children, 47 were classified as (a) NSRW, 78 as (b) SRW, 108 as (c) NSA, and 62 as (d) SA (Fig 1). Their main characteristics are presented in Table 1. Children with SRW had significantly lower birth weight than those with NSRW (3100g [2600, 3430] vs 3380g [3102, 3550], p=0.004) and were more frequently exposed to second-hand smoke (22.7% vs 2.1%, p=0.004) and visible mold/dampness (28.2% vs 8.7%, p=0.019). Children with SA had a more frequent history of FA (26.7% vs 13.2%, p=0.05) and atopic dermatitis (AD) (46.8% vs 26.9%, p=0.014) than those with NSA. Atopy was found in 61.3% of preschool children and 82.2% of school-age children, without differences between NSRW and SRW or NSA and SA, respectively.

Sensitization profile differences between non-severe and severe patients

Overall, we observed individual c-sIgE sensitization (at least one positive c-sIgE ≥0.30 ISU) for 51.4% of preschool children and 75.3% of school-age children.

Among preschool children, at the biological source level, 21.5% were sensitized to at least one food, 45.9% to at least one airborne, and 20% to at least one cross-reactive allergen (Table 2). Preschool children with NSRW more frequently had multi-sensitization (≥2 biological sources) than those with SRW (51.1% vs 24.4%, p=0.002), although there was no difference for food and cross-reactive components. Airborne allergen and house dust mite (HDM) sensitizations were more frequent among children with NSRW than SRW (60.9% vs 36.8%; p=0.010) and 53.2% vs 24.4% (p=0.001), respectively. At the component level, patterns of sensitization to individual allergen components did not clearly discriminate NSRW from SRW or NSA from SA (Figure 2). However, sensitization (concentration ≥0.30 ISU) to the HDM components Der f...
1 (38.3% vs 26.4%, p=0.032), Der f 2 (42.6% vs 16.7%, p=0.003), Der p 1 (42.6% vs 19.2%, p=0.009), and Der p 2 (48.9% vs 17.9%, p<0.001) was more frequent among children with NSA than SA (Sup Table 1). There was no difference in terms of c-sIgE components [?]15 ISU (Sup Table 2).

Among school-age children, at the biological source level, 23.7% were sensitized to at least one food, 74.1% to at least one airborne, and 32.1% to at least one cross-reactive allergen (Table 2). The rates of multi-sensitization were comparable between children with NSA and SA (62% vs 61.3%, p=0.92). There was no difference in airborne sensitization profiles but sensitization to non-specific lipid transfer protein (nsLTP) was more frequent among children with SA than NSA (16.1% vs 3.7%, p=0.005). At the component level, the number of children with c-sIgE [?]0.30 ISU did not differ between children with SA and NSA, except for the food components Gal d 1/ovomucoid (6.5% vs 0%, p=0.032) and Cor a 9 (8.1% vs 0.9%, p=0.046), the airborne components Can f 1 (22.6% vs 8.3%, p=0.017), Can f 2 (14.5% vs 2.8%, p=0.01), and the nsLTP components Art v 3 (8.1% vs 0.9%, p=0.046) and Cor a 8 (6.5% vs 0%, p=0.032) (Sup Table 1). The number of children with c-sIgE [?]15 ISU was comparable between SA and NSA (Sup Table 2).

Age and sensitization profiles
We observed an increase in the numbers and levels of c-sIgE sensitization with age, both among non-severe and severe patients (Fig 2). In particular, there was an increase in the number of positive biological sources for airborne (RR 1.14 [1.08 – 1.20], p<0.0001) and cross-reactive components (RR 1.18 [1.07 – 1.30] per one-year increase in age, p=0.00098), but not for food biological sources (Sup Fig 2, Sup Table 3).

Lung function and sensitization profiles
Among the 235 participants with available data on lung function, there was no significant relationship between c-sIgE sensitization and the forced expiratory volume in one second (FEV1) / forced vital capacity (FVC) z-score, except for the frequency of nsLTP sensitization, which was higher for the participants with a FEV1/FVC z-score < -1.64 than in the others (16.7% vs 5.2%, p=0.017) (Sup Table 4).

Supervised multivariate analysis
Principal component analysis (PCA) was performed with the c-IgE values for the preschool children. PC1 accounted for 20.3% of the variance and PC2 for 11.7%. Overall, PCA did not allow differentiation between NSRW and SRW. Random forest analysis was then performed. Similarly, it did not allow discrimination between NSRW and SRW, with an estimated out-of-bag error rate of 43.1% and a ROC AUC of 0.56 (Sup Fig 3).

Among school-age children, PCA, with PC1 explaining 24.1% of the variance and PC2 10.4%, did not allow differentiation between NSA and SA. Similarly, random forest analysis did not allow discrimination between NSA and SA, with an estimated out of bag error rate of 33.9% and a ROC AUC of 0.53 (Sup Fig 4).

Unsupervised Clustering of children with positive c-sIgE
Among preschool children with at least one positive c-sIgE (n=61), three clusters (clusters 1-3) of different size were generated: Cluster 1 (C1, n=4, 6.6%), with “multiple sensitizations, mainly to grass pollens and pathogenesis–related protein family 10 (PR-10)”, Cluster 2 (C2, n=4, 6.6%), with “multiple sensitizations, mainly to food, grass pollens, animal dander, and nsLTP”, and Cluster 3 (C3, n=53, 86.9%), with “few sensitizations, mainly to HDM” (Sup Fig 5). The distribution of SRW within the three clusters did not differ, but three of the four patients of Cluster 2 had SRW. Overall, lung function parameters were similar between the three clusters (Table 3).

Among school-age children with positive c-sIgE (n=128), four clusters (clusters 4-7) were generated: Cluster 4 (n=4, 3.1%), with “multiple sensitizations, mainly to grass pollens, HDM, PR-10, and nsLTP”, Cluster 5 (n=6, 4.7%) with “multiple sensitizations, mainly to airborne allergens, including grass pollens and HDM”, Cluster 6 (n=24, 18.8%), with “multiple sensitizations, mainly to grass pollens, HDM, and PR-10”, and Cluster 7 (n=94, 73.4%) with “few sensitizations, mainly to HDM” (Sup Fig 6). All four patients from
Cluster 4 had SA, vs 33% in Cluster 5, 25% in Cluster 6, and 34% in Cluster 7 (p=0.036). Lung function parameters were comparable between the four clusters (Table 4, Sup Table 5).

Discussion

1/ Main results

In this study, we aimed to determine whether sensitization profiles for children with SRW or SA could be distinguishable from those with NSRW or NSA using a CRD multiplex assay. Although c-sIgE sensitization was frequent, observed in 51% of preschoolers and 75% of school-age children, the patterns of biological source sensitization did not clearly discriminate between preschool children with NSRW and those with SRW or school-age children with NSA and those with SA. At the component level, we observed age-related specificities. Sensitization to airborne allergens, especially towards HDM components, and multi-sensitization, were approximately twice as frequent among preschoolers with NSRW than those with SRW. At school-age, sensitization to some components (ovomucoid, hazelnut 2 S globulin, dog salivary lipocalin proteins, and nsLTP) was more frequent among children with SA and sensitization to nsLTP was associated with impaired lung function. Unsupervised clustering confirmed the heterogeneity in sensitization profiles, identifying three clusters for preschool children and four for school-age children, of different sizes, with shared patterns but also some specificities (grass and PR-10 among preschool children and nsLTP among school-age children). Only one small cluster with multiple airborne and nsLTP sensitization was associated with asthma severity at school-age.

2/ Most sensitized children with recurrent wheeze/asthma show comparable patterns, with a predominance of HDM sensitization

Sensitization was observed in more than half of preschoolers, confirming that sensitization may appear early during the course of childhood asthma. Interestingly, although preschoolers were less frequently sensitized than school-age children, the sensitization profiles in the two age-groups showed strong similarities. The two broader clusters identified in each age-group were characterized by few sensitizations, mainly to HDM, and were comparable to clusters previously described in the U-BIOPRED cohort, although in this cohort, only 27% of preschoolers were sensitized (10). Sensitization to HDM and multi-sensitization were even more frequent among preschoolers with NSRW than those with SRW, supporting our previous findings that disease severity is associated with exposure to mold and cigarette smoke rather than atopy in this age-group (11).

The finding that patterns of sensitization to biological sources did not clearly discriminate between children with SA/SRW and those with milder disease confirm the results from the U-BIOPRED cohort (10). As already mentioned, despite preschoolers being less frequently sensitized than school-age children, the profile of sensitization of children from the two groups showed strong similarities, with the two broader clusters identified for children from each group being characterized by few sensitizations, mainly to HDM. This would suggest that, at least among sensitized children, asthma in school-age children may share common features with wheezing in preschoolers.

Overall, these results confirm that sensitization patterns may not be useful biomarkers of disease severity in children when described in terms of numbers/levels of component sensitization at a single time point, as previously described (10). Our results reinforce the hypothesis that more complex endotypic mechanisms, rather than simple allergenic sensitization, underpin asthma severity during childhood.

3/ Minor groups of children are characterized by distinct patterns, with sensitization to pan-allergens

Our cluster analysis found that some preschool and school-age children showed multiple sensitization, including sensitization to pan-allergens. Two small clusters were characterized by sensitization to PR-10 proteins, a super-family of highly conserved pan-allergens, previously shown to be associated with asthma and allergic rhinitis at school-age (13,14,16,17). In particular, Bet v 1 was identified to be one of the five allergen molecules associated with a high risk of asthma and/or rhinitis at 16 years of age in two North European birth cohorts (18). More broadly, studies conducted in birch-free areas have suggested that sensitization
to PR-10 proteins is associated with clinical phenotypes and sensitization to other allergens (19,20). Our study confirms these observations by identifying one of the PR-10 clusters in preschool recurrent wheezers, suggesting that children developing early sensitization to these pan-allergens may constitute a distinct endotype. These results also complement the findings from the network analysis of component sensitization among children from the U-BIOPRED cohort, showing that patients with SA had more frequent connections between components than those with milder disease (10).

4/ Sensitization to certain single components is associated with severity, in particular to nsLTP

In the present study, sensitization to the food components Gal d 1 (ovomucoid) and Cor a 9 (11S seed-storageglobulin) and the airborne dog allergens Can f 1 and Can f 2 (lipocalins) was more frequent among school-age children with SA than those with NSA. These results confirm recent findings that Can f 2 sensitization and multi-sensitization to lipocalins are more frequent among children with SA than those with milder disease (21,22). Interestingly, sensitization to the nsLTPs Cor a 8 and Art v 3 was associated with SA, and sensitization to Pru p 3, a major nsLTP, also tended to be more frequent among SA. In addition, nsLTP sensitization was associated with lower lung function as assessed by the FEV1/FVC < -1.64 z-score. Furthermore, in contrast to the multi-sensitization pattern shown in cluster 5, nsLTP sensitization was a characteristic of the sensitization profile shown in cluster 4, which was the only cluster associated with SA. Among preschoolers, 75% of children from cluster 2, also characterized by nsLTP sensitization, had SRW. This association of nsLTP sensitization with asthma severity has not been described elsewhere. This may result, at least partially, from the high geographical variation in the prevalence of nsLTP sensitization (23). nsLTP have been reported to be the most common food allergens associated with anaphylaxis in Italy and Spain. In parallel with global climate change and the rising spread and allergenicity of pollens, pediatric cases of nsLTP sensitization are increasing (24,25). Sensitization towards nsLTP from pollen and food were observed in this cohort. It is yet to be determined whether sensitization to nsLTP primarily occurs through pollen or food exposure (24). Although these results need confirmation, in particular because of the small size of cluster 4, they highlight how geographical variation might affect asthma severity.

4. The longitudinal follow-up of the cohort will allow the comparison of sensitization patterns as biomarkers of disease trajectories

As expected, we observed an increase in sensitization in terms of the number of positive c-sIgE and levels of sensitization between the age of 3 and 12 years. Early and multiple sensitizations, in particular to the airborne allergens HDM and grass pollen, are risk factors for the persistence of asthma, recurrence, severity of attacks and long-term lung function impairment (5–9). We did not observe any relationship between mold sensitization and SRW / SA. This is in contrast with other studies showing an association between mold exposure, mold sensitization and asthma exacerbations and/or severe asthma (26–28). However, Mold sensitization was retained in only a limited number of children in our study, 9 preschoolers and 15 school-age children, which did not allow full exploration of its association with severity because of lack of power. The follow-up of this cohort will make it possible to analyze sensitization trajectories and provide new insights into the natural course of sensitization. The pathophysiological mechanisms linking sensitization patterns to persistent and/or severe asthma, with altered lung function, may include an unbalanced immune reaction biased toward a response involving type 2 helper T cells (Th2) in children with early and multiple occurrences of sensitization (9,29) and exacerbated interferon production in response to viral infections in children with late-onset sensitization and asthma (29).

5. Strengths and limitations

The French CobraPed cohort has enrolled a subsequent and well-characterized population. In particular, preschoolers represent a significant number, of whom 61 could be included in the cluster analysis on the basis of positivity to at least one c-sIgE, providing more information than previously (10). However, this study had several limitations. We excluded patients receiving the anti-IgE omalizumab. Although the biological rationale behind this choice is clear, we excluded severe asthmatic patients, often highly atopic with multiple sensitizations (30,31). However, because omalizumab was mostly offered to school-age children,
per guidelines, the results for the preschoolers was not influenced by this bias. In addition, we applied a strict definition for SRW and SA based on the current guidelines at the time of enrollment (12). Consequently, the patients with SRW/SA had a high burden, with frequent symptoms, exacerbations, and alteration of lung function and were representative of these children.

Conclusion

Sensitization was frequently observed among children included in the French CobraPed cohort, even among preschoolers. However, sensitization patterns did not clearly discriminate between children with severe and non-severe disease, suggesting that other mechanisms underpin asthma severity.

Funding information

This study was funded by the “Chancellerie des Universités de Paris (legs Gaston Poix)” and grants from GSK, Stallergènes, Chiesi, Novartis, and Mundi Pharma Laboratories.

Conflict of interests

In the past 3 years, SL declares having received research grants from Astra Zeneca and Santelys, remunerations for symposiums by Sanofi and Novartis.

FA declares personal fees for consulting, lectures or boards from Stallergenes Greer, ALK, AImmune Therapeutics, GSK, Novartis, and Sanofi.

AD declares consultancy or speaker fees from Novartis, GSK, Sanofi, Regeneron, AstraZeneca, Aimmune Therapeutics, DBV Technologies, Nestlé Health Science, ALK, Stallergènes-Greer outside the submitted work.

GL declares remunerations for symposia from DBV Technologies, Aimmune Therapeutics, for conferences from Novartis Pharma, Astra Zeneca, ALK, board consulting for ALK, Stallergènes-Greer, Aimmune Therapeutics, advices for Meenuts, expert consulting for ALK, Stallergenes-Greer.

Authors contributions

Conceptualization: SL, NB, JdB, AD, GL
Methodology: SL, NB, PRN, VJ, JdB, AD, GL
Software: NB, VJ, PRN
Formal analysis: SL, NB, AD, GL
Visualization: SL, NB
Funding acquisition: JdB, AD, GL
Project administration: JdB, GL
Supervision: JdB, AD, GL
Writing – original draft: SL, NB, AD, GL

References


36. Dreborg S. Skin testing. The safety of skin tests and the information obtained from using different methods and concentrations of allergen. *Allergy* 1993;48:473–475.


Table 1. Description of the study population

<table>
<thead>
<tr>
<th>Demographics</th>
<th>Age, y</th>
<th>Male gender</th>
<th>Caucasian</th>
<th>Birth weight, g</th>
<th>Z-score for BMI</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>5 [4, 6]</td>
<td>84/125 (67.2)</td>
<td>91/119 (76.5)</td>
<td>3185 [2800, 3502]</td>
<td>2.0 [-0.4, 1.7]</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Environment</th>
<th>Rural or semi-rural home</th>
<th>Mother smoked during pregnancy</th>
<th>Second-hand smoke exposure</th>
<th>Visible mold/dampness at home</th>
<th>Pet</th>
<th>History of food allergy</th>
<th>History of allergic rhinitis</th>
<th>History of atopic dermatitis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Associated allergic disorders</th>
<th>Family atopy in 1 parent or sibling</th>
<th>History of food allergy</th>
<th>History of allergic rhinitis</th>
<th>History of atopic dermatitis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Asthma outcomes</th>
<th>Age at first wheeze (months)</th>
<th>No. of admissions for asthma exacerbation in childhood</th>
<th>Asthma exacerbation in the previous year</th>
<th>ACT score</th>
<th>Total PAQLQ score</th>
<th>FEV1 pre-BD (Z-score)</th>
<th>FEV1/FVC pre-BD (Z-score)</th>
<th>ICS</th>
<th>ICS doses (μg/d eq Budesonide)</th>
<th>ICS + LABA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>5 [2, 8]</td>
<td>n'=104</td>
<td>1 [0, 4]</td>
<td>71/123 (57.7)</td>
<td>20 [16, 23]</td>
<td>-0.1 [-0.9, 0.9]</td>
<td>-0.3 [-1.3, 0.9]</td>
<td>5</td>
<td>78/78 (100.0)</td>
<td>1000 [800, 1837]</td>
</tr>
</tbody>
</table>

Table 2. Differences in the sensitization profile (biological source) between (a) NSRW and (b) SRW and between (c) NSA and (d) SA

<table>
<thead>
<tr>
<th>Total preschool population (N = 125)</th>
<th>(a) NSRW (n = 47)</th>
<th>(b) SRW (n = 78)</th>
<th>p-value (a) vs (b)</th>
<th>Total school-age population (n = 170)</th>
<th>(c) NSA (n = 108)</th>
<th>(d) SA (n = 62)</th>
<th>p-value (c) vs (d)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No sensitization</td>
<td>61 (49.6%)</td>
<td>17 (36.2%)</td>
<td>44</td>
<td>0.019</td>
<td>42 (24.7%)</td>
<td>24 (22.2%)</td>
<td>18</td>
</tr>
<tr>
<td>Number of positive c-sIgE determinants</td>
<td>6 [3-10]</td>
<td>6 [3.3-]</td>
<td>5.5</td>
<td>0.44</td>
<td>7 [5-14]</td>
<td>7 [5-12]</td>
<td>17</td>
</tr>
<tr>
<td>of positive c-sIgE determinants</td>
<td>19 (15.2%)</td>
<td>11.5 (12.8%)</td>
<td>6 [2.0-7.8]</td>
<td>0.0023</td>
<td>23 (13.5%)</td>
<td>[4.8-15]</td>
<td>17 [15.7%]</td>
</tr>
<tr>
<td>Monosensitization</td>
<td>65/106 (61.3%)</td>
<td>89 [28.2, 317.2]</td>
<td>250 [120, 500]</td>
<td>0.57</td>
<td>676.8 [200, 600]</td>
<td>239 [74.0, 811.5]</td>
<td>400 [195.5, 800.0]</td>
</tr>
<tr>
<td>Total</td>
<td>0.44</td>
<td>0.56</td>
<td>0.0023</td>
<td>0.32</td>
<td>0.48</td>
<td>0.27</td>
<td>0.92</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Food</th>
<th>Total preschool population (N = 125)</th>
<th>(a) NSRW (n = 47)</th>
<th>(b) SRW (n = 78)</th>
<th>p-value (a) vs (b)</th>
<th>Total school-age population (n = 170)</th>
<th>(c) NSA (n = 108)</th>
<th>(d) SA (n = 62)</th>
<th>p-value (c) vs (d)</th>
</tr>
</thead>
<tbody>
<tr>
<td>biological</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>egg</td>
<td>(21.5%) 26</td>
<td>(25.5%) 12</td>
<td>(18.9%) 14</td>
<td>0.39</td>
<td>(23.7%) 40</td>
<td>(23.1%) 25</td>
<td>(24.6%) 15</td>
<td>0.83</td>
</tr>
<tr>
<td>cow’s</td>
<td>(1.6%) 5</td>
<td>(2.1%) 1</td>
<td>(1.3%) 1</td>
<td>0.99</td>
<td>(2.9%) 5</td>
<td>(0.9%) 1</td>
<td>(4.6%) 4</td>
<td>0.14</td>
</tr>
<tr>
<td>shrimp</td>
<td>(10%) 12</td>
<td>(10.6%) 5</td>
<td>(6.4%) 7</td>
<td>0.99</td>
<td>(12.4%) 21</td>
<td>(9.3%) 7</td>
<td>(18%) 9</td>
<td>0.99</td>
</tr>
<tr>
<td>nuts</td>
<td>(6.4%) 10</td>
<td>(4.3%) 2</td>
<td>(1.3%) 1</td>
<td>0.63</td>
<td>(6.5%) 16</td>
<td>(6.5%) 2</td>
<td>(14.5%) 11</td>
<td>0.99</td>
</tr>
<tr>
<td>legumes</td>
<td>(0.8%) 12</td>
<td>(0%) 0</td>
<td>(2.6%) 2</td>
<td></td>
<td>(9.4%) 12</td>
<td>(1.9%) 7</td>
<td>(1.6%) 1</td>
<td></td>
</tr>
<tr>
<td>cereals</td>
<td>(3.2%) 10</td>
<td>(4.3%) 2</td>
<td>(9.5%) 7</td>
<td></td>
<td>(1.8%) 10</td>
<td>(6.5%) 4</td>
<td>(6.5%) 4</td>
<td></td>
</tr>
<tr>
<td>fruit</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Airborne</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>biological</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pollen</td>
<td>(45.9%) 56</td>
<td>(60.9%) 28</td>
<td>(36.8%) 26</td>
<td>0.0098</td>
<td>(74.1%) 126</td>
<td>(75.9%) 82</td>
<td>(71%) 44</td>
<td>0.48</td>
</tr>
<tr>
<td>source</td>
<td>(2.16%) 22</td>
<td>(4.3%) 11</td>
<td>(0%) 11</td>
<td>0.065</td>
<td>(0.6%) 66</td>
<td>(0%) 42</td>
<td>(1.6%) 24</td>
<td>0.98</td>
</tr>
<tr>
<td>Polcal-</td>
<td>(17.6%) 15</td>
<td>(23.4%) 9</td>
<td>(14.1%) 6</td>
<td>0.20</td>
<td>(38.8%) 52</td>
<td>(38.9%) 34</td>
<td>(38.7%) 18</td>
<td>0.69</td>
</tr>
<tr>
<td>Grass</td>
<td>(12.5%) 22</td>
<td>(19.6%) 12</td>
<td>(2.6%) 6</td>
<td>0.0011</td>
<td>(30.8%) 52</td>
<td>(31.5%) 34</td>
<td>(29.5%) 18</td>
<td>0.79</td>
</tr>
<tr>
<td>pollen</td>
<td>(4.8%) 6</td>
<td>(8.5%) 4</td>
<td>(8.5%) 10</td>
<td></td>
<td>(14.7%) 25</td>
<td>(13.9%) 15</td>
<td>(16.1%) 10</td>
<td>0.47</td>
</tr>
<tr>
<td>Tree</td>
<td>(17.6%) 22</td>
<td>(25.5%) 6</td>
<td>(7.7%) 6</td>
<td>0.52</td>
<td>(38.8%) 66</td>
<td>(38%) 41</td>
<td>(40.3%) 25</td>
<td>0.46</td>
</tr>
<tr>
<td>pollen</td>
<td>(7.2%) 9</td>
<td>(6.4%) 3</td>
<td>(6.4%) 19</td>
<td></td>
<td>(38.8%) 15</td>
<td>(38%) 10</td>
<td>(5.81%) 5</td>
<td>0.36 -</td>
</tr>
<tr>
<td>Animals</td>
<td>(35.2%) 44</td>
<td>(53.2%) 25</td>
<td>(24.4%) 15</td>
<td></td>
<td>(8.8%) 102</td>
<td>(9.3%) 67</td>
<td>(56.5%) 6</td>
<td></td>
</tr>
<tr>
<td>Mold</td>
<td>(2.16%) 9</td>
<td>(4.3%) 2</td>
<td>(2.7%) 0</td>
<td></td>
<td>(60%) 62</td>
<td>(62%) 42</td>
<td>(3.2%) 2</td>
<td></td>
</tr>
<tr>
<td>HDM</td>
<td>(1.7%) 2</td>
<td>(0%) 0</td>
<td>(0%) 0</td>
<td></td>
<td>(3.5%) 8</td>
<td>(3.7%) 4</td>
<td>(4.6%) 4</td>
<td></td>
</tr>
<tr>
<td>Insects</td>
<td>(0%) 0</td>
<td>(0%) 0</td>
<td>(0%) 0</td>
<td></td>
<td>(4.7%) 1</td>
<td>(3.7%) 0</td>
<td>(1.6%) 0</td>
<td></td>
</tr>
<tr>
<td>Venom</td>
<td>(0%) 0</td>
<td>(0%) 0</td>
<td>(0%) 0</td>
<td></td>
<td>(6.0%) 0</td>
<td>(0%) 0</td>
<td>(0%) 0</td>
<td></td>
</tr>
<tr>
<td>Parasite</td>
<td>(0%) 0</td>
<td>(0%) 0</td>
<td>(0%) 0</td>
<td></td>
<td>(0%) 0</td>
<td>(0%) 0</td>
<td>(0%) 0</td>
<td></td>
</tr>
</tbody>
</table>

*p-values were calculated using Fisher’s exact test (two-tailed) where *denotes significant differences*.
<table>
<thead>
<tr>
<th></th>
<th>Total preschool population (N = 125)</th>
<th>Total school-age population (n = 170)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(a) NSRW (n = 47)</td>
<td>(c) NSA (n = 108)</td>
</tr>
<tr>
<td></td>
<td>(b) SRW (n = 78)</td>
<td>(d) SA (n = 62)</td>
</tr>
<tr>
<td></td>
<td>p-value (a) vs (b)</td>
<td>p-value (c) vs (d)</td>
</tr>
<tr>
<td><strong>Cross-Reactive</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Allergens (20%)</td>
<td>25</td>
<td>56</td>
</tr>
<tr>
<td>(8.8%) 5</td>
<td>11</td>
<td>(32.9%)</td>
</tr>
<tr>
<td>Tropomyosin (4%)</td>
<td>25</td>
<td>11</td>
</tr>
<tr>
<td>(5.1%) 4</td>
<td>13</td>
<td>(6.5%)</td>
</tr>
<tr>
<td>Serum (4%) 6</td>
<td>11</td>
<td>4 (3.7%)</td>
</tr>
<tr>
<td>Protein (4.8%)</td>
<td>3</td>
<td>23</td>
</tr>
<tr>
<td>nsLTP (2.4%) 2</td>
<td>13</td>
<td>15</td>
</tr>
<tr>
<td>PR-10 (1.6%) 5</td>
<td>12</td>
<td>9 (22.4%)</td>
</tr>
<tr>
<td>TLP (4%)</td>
<td>3</td>
<td>3 (6.4%)</td>
</tr>
<tr>
<td>Profilin</td>
<td>2</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>CCD</td>
<td>13</td>
<td>3 (7.6%)</td>
</tr>
</tbody>
</table>

**c-sIgE were dichotomized using a binary threshold (positive = 0.30 ISU)**

**CCD**: cross-reactive carbohydrate determinants, **HDM**: house dust mite, **NSA**: non-severe school-age asthmatic children, **nsLTP**: non-specific lipid transfer protein, **NSRW**: non-severe preschool recurrent wheezers, **PR-10**: pathogenesis-related protein family 10, **SRW**: severe preschool recurrent wheezers, **TLP**: thaumatin-like proteins

**Table 3. Severity and lung function by cluster in preschool children**

<table>
<thead>
<tr>
<th>Cluster 1</th>
<th>Cluster 2</th>
<th>Cluster 3</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Multiple, mainly grass pollens and PR-10 n = 4</td>
<td>Multiple, mainly food, grass pollens, animal dander and nsLTP N = 4</td>
<td>Few, mainly HDM n = 53</td>
<td></td>
</tr>
<tr>
<td>Severe recurrent wheeze</td>
<td>1 (25%)</td>
<td>3 (75%)</td>
<td>27 (51%)</td>
</tr>
<tr>
<td>Z-score FEV1</td>
<td>n’=2 -0.10 [-0.49, 0.29]</td>
<td>n’=3 - 0.59 [-0.65, -0.34]</td>
<td>n’=38 0.015 [-0.51, 0.86]</td>
</tr>
<tr>
<td>Z-score FEV1/FVC</td>
<td>-0.63 [-0.72, -0.53]</td>
<td>-1.36 [-1.5, -0.95]</td>
<td>0.010 [-1.13, 0.64]</td>
</tr>
</tbody>
</table>

**FEV1**: forced expiratory volume in one second, **FVC**: forced vital capacity, **HDM**: house dust mite, **PR-10**: pathogenesis-related protein family 10

**Table 4. Severity and lung function by cluster in school-age children**
<table>
<thead>
<tr>
<th>Cluster 4</th>
<th>Cluster 5</th>
<th>Cluster 6</th>
<th>Cluster 7</th>
</tr>
</thead>
<tbody>
<tr>
<td>Multiple, mainly grass pollens, HDM, PR-10 and nsLTP n = 4</td>
<td>Multiple, mainly airborne including grass pollens and HDM n = 6</td>
<td>Multiple, mainly grass pollens, HDM and PR-10 N = 24</td>
<td>Few, mainly HDM n = 94</td>
</tr>
<tr>
<td>Severe asthma</td>
<td>Z-score FEV1</td>
<td>Z-score</td>
<td>Z-score</td>
</tr>
<tr>
<td>4 (100%)</td>
<td>n'=4 -0.89 [-1.92, 0.078]</td>
<td>-1.69 [-2.1, -0.25]</td>
<td>-0.67 [-1.48, 0.18]</td>
</tr>
<tr>
<td>2 (33%)</td>
<td>n'=6 0.6 [0.35, 0.92]</td>
<td>-0.92 [-1.33, -1.37]</td>
<td>-0.67 [-1.48, 0.18]</td>
</tr>
<tr>
<td>6 (25%)</td>
<td>n'=23 0.08 [-0.87, 0.93]</td>
<td>-0.44 [-1.37, 0.11]</td>
<td>-0.67 [-1.48, 0.18]</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>0.036</td>
</tr>
</tbody>
</table>

FEV1: forced expiratory volume in one second, FVC: forced vital capacity, HDM: house dust mite, PR-10: pathogenesis-related protein family 10

Figure 1. Flow-chart of the study population


Figure 2. Patterns of sensitization to each allergen component (columns) for individual participants (rows) stratified by severity group

Hosted file