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

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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.

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Short Title: Allergen sensitization in the COBRAPed cohort

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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

ISAC: ImmunoCAP Immuno Solid-phase Allergen Chip

ISU: ImmunoCAP Immuno Solid-phase Allergen Chip Standardized Units

LABA: long-acting beta-agonists

NSA: non-severe school-age asthmatic children

nsLTP: non-specific lipid transfer protein

NSRW: non-severe preschool recurrent wheezers

PAQLQ: Pediatric Asthma Quality of Life Score

PR-10: pathogenesis-related protein family 10

SA: severe school-age asthmatic children

SABA: short-acting β-agonist

SPT: skin-prick test

SRW: severe preschool recurrent wheezers

TLP: thaumatin-like proteins

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 tor 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.

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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, Stallergenes-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

Investigation: SL, VJ, LR, CM, FA, PB, MF, JCD, SV, PR, MP, JB, RC, LGC, 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

1. Lang A, Carlsen KH, Haaland G, Devulapalli CS, Munthe-Kaas M, Mowinckel P et al. Severe asthma in childhood: assessed in 10 year olds in a birth cohort study. *Allergy* 2008;**63** :1054–1060.

2. Chung KF, Wenzel SE, Brozek JL, Bush A, Castro M, Sterk PJ et al. International ERS/ATS guidelines on definition, evaluation and treatment of severe asthma. *Eur Respir J* 2014;**43** :343–373.

3. Fitzpatrick AM, Teague WG, Meyers DA, Peters SP, Li X, Li H et al. Heterogeneity of severe asthma in childhood: confirmation by cluster analysis of children in the National Institutes of Health/National Heart, Lung, and Blood Institute Severe Asthma Research Program. *J Allergy Clin Immunol* 2011;**127** :382-389.e1-13.

4. Just J, Bourgoin-Heck M, Amat F. Clinical phenotypes in asthma during childhood. *Clin Exp Allergy* 2017;**47** :848–855.

5. Custovic A, Sonntag H-J, Buchan IE, Belgrave D, Simpson A, Prosperi MCF. Evolution pathways of IgE responses to grass and mite allergens throughout childhood. *J Allergy Clin Immunol*2015;**136** :1645-1652.e8.

6. Posa D, Perna S, Resch Y, Lupinek C, Panetta V, Hofmaier S et al. Evolution and predictive value of IgE responses toward a comprehensive panel of house dust mite allergens during the first 2 decades of life.*J Allergy Clin Immunol* 2017;**139** :541-549.e8.

7. Illi S, von Mutius E, Lau S, Niggemann B, Grüber C, Wahn U et al. Perennial allergen sensitisation early in life and chronic asthma in children: a birth cohort study. *Lancet*2006;**368** :763–770.

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. 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.e12.

10. Roberts G, Fontanella S, Selby A, Howard R, Filippi S, Hedlin G et al. Connectivity patterns between multiple allergen specific IgE antibodies and their association with severe asthma. *J Allergy Clin Immunol* 2020;**146** :821–830.

11. Lezmi G, Lejeune S, Pin I, Blanchon S, Bouazza N, Jolaine V et al. Factors Associated with Asthma Severity in Children: Data from the French COBRAPed Cohort. *J Allergy Clin Immunol Pract*2021;**9** :1969–1979.

12. Hedlin G, Bush A, Lødrup Carlsen K, Wennergren G, De Benedictis FM, Melén E et al. Problematic severe asthma in children, not one problem but many: a GA2LEN initiative. *Eur Respir J*2010;**36** :196–201.

13. Fontanella S, Frainay C, Murray CS, Simpson A, Custovic A. Machine learning to identify pairwise interactions between specific IgE antibodies and their association with asthma: A cross-sectional analysis within a population-based birth cohort. *PLOS Medicine*2018;**15** :e1002691.

14. Howard R, Belgrave D, Papastamoulis P, Simpson A, Rattray M, Custovic A. Evolution of IgE responses to multiple allergen components throughout childhood. *Journal of Allergy and Clinical Immunology*2018;**142** :1322–1330.

15. Papastamoulis P, Rattray M. BayesBinMix: an R Package for Model Based Clustering of Multivariate Binary Data. *The R Journal*2017;**9** :403.

16. Loraud C, de Ménonville CT, Bourgoin-Heck M, Cottel N, Wanin S, Just J. Emergence of pollen food allergy syndrome in asthmatic children in Paris. *Pediatr Allergy Immunol* 2021;**32** :702–708.

17. 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.e1-11.

18. 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;**26** :91–99.

19. Ciprandi G, Silvestri M, Pistorio A, Olcese R, Del Barba P, Tosca MA. Bet v 1 sensitization modulates allergenic molecular immune response. *Eur Ann Allergy Clin Immunol* 2019;**51** :21–31.

20. Scala E, Abeni D, Cecchi L, Guerra EC, Locanto M, Pirrotta L et al. Molecular Recognition Profiles and Clinical Patterns of PR-10 Sensitization in a Birch-Free Mediterranean Area. *Int Arch Allergy Immunol* 2017;**173** :138–146.

21. Konradsen JR, Nordlund B, Onell A, Borres MP, Grönlund H, Hedlin G. Severe childhood asthma and allergy to furry animals: refined assessment using molecular-based allergy diagnostics. *Pediatr Allergy Immunol* 2014;**25** :187–192.

22. Käck U, van Hage M, Grönlund H, Lilja G, Asarnoj A, Konradsen JR. Allergic sensitization to lipocalins reflects asthma morbidity in dog dander sensitized children. *Clin Transl Allergy*2022;**12** :e12149.

23. Asero R, Antonicelli L, Arena A, Bommarito L, Caruso B, Crivellaro M et al. EpidemAAITO: features of food allergy in Italian adults attending allergy clinics: a multi-centre study. *Clin Exp Allergy*2009;**39** :547–555.

24. Olivieri B, Stoenchev KV, Skypala IJ. Anaphylaxis across Europe: are pollen food syndrome and lipid transfer protein allergy so far apart?*Curr Opin Allergy Clin Immunol* 2022;**22** :291–297.

25. Mastrorilli C, Tripodi S, Caffarelli C, Perna S, Di Rienzo-Businco A, Sfika I et al. Endotypes of pollen-food syndrome in children with seasonal allergic rhinoconjunctivitis: a molecular classification.*Allergy* 2016;**71** :1181–1191.

26. Caillaud D, Leynaert B, Keirsbulck M, Nadif R, mould ANSES working group. Indoor mould exposure, asthma and rhinitis: findings from systematic reviews and recent longitudinal studies. *Eur Respir Rev* 2018;**27** :170137.

27. Bush A. Kids, Difficult Asthma and Fungus. *J Fungi (Basel)*2020;**6** :55.

28. Kanchongkittiphon W, Mendell MJ, Gaffin JM, Wang G, Phipatanakul W. Indoor environmental exposures and exacerbation of asthma: an update to the 2000 review by the Institute of Medicine. *Environ Health Perspect* 2015;**123** :6–20.

29. Custovic A, Belgrave D, Lin L, Bakhsoliani E, Telcian AG, Solari R et al. Cytokine Responses to Rhinovirus and Development of Asthma, Allergic Sensitization, and Respiratory Infections during Childhood.*Am J Respir Crit Care Med* 2018;**197** :1265–1274.

30. Deschildre A, Marguet C, Salleron J, Pin I, Rittié J-L, Derelle J et al. Add-on omalizumab in children with severe allergic asthma: a 1-year real life survey. *Eur Respir J* 2013;**42** :1224–1233.

31. Lieberman PL, Umetsu DT, Carrigan GJ, Rahmaoui A. Anaphylactic reactions associated with omalizumab administration: Analysis of a case-control study. *J Allergy Clin Immunol*2016;**138** :913-915.e2.

32. Rolland-Cachera MF, Sempé M, Guilloud-Bataille M, Patois E, Péquignot-Guggenbuhl F, Fautrad V. Adiposity indices in children.*Am J Clin Nutr* 1982;**36** :178–184.

33. Nathan RA, Sorkness CA, Kosinski M, Schatz M, Li JT, Marcus P et al. Development of the asthma control test: a survey for assessing asthma control. *J Allergy Clin Immunol* 2004;**113** :59–65.

34. Liu AH, Zeiger R, Sorkness C, Mahr T, Ostrom N, Burgess S et al. Development and cross-sectional validation of the Childhood Asthma Control Test. *J Allergy Clin Immunol* 2007;**119** :817–825.

35. Juniper EF, Guyatt GH, Feeny DH, Ferrie PJ, Griffith LE, Townsend M. Measuring quality of life in children with asthma. *Qual Life Res*1996;**5** :35–46.

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.

37. Miller MR, Hankinson J, Brusasco V, Burgos F, Casaburi R, Coates A et al. Standardisation of spirometry. *Eur Respir J*2005;**26** :319–338.

38. Beydon N, Davis SD, Lombardi E, Allen JL, Arets HGM, Aurora P et al. An official American Thoracic Society/European Respiratory Society statement: pulmonary function testing in preschool children. *Am J Respir Crit Care Med* 2007;**175** :1304–1345.

39. Stanojevic S, Kaminsky DA, Miller MR, Thompson B, Aliverti A, Barjaktarevic I et al. ERS/ATS technical standard on interpretive strategies for routine lung function tests. *Eur Respir J*2022;**60** :2101499.

Table 1. Description of the study population

Demographics Age, y Male gender Caucasian Birth weight, g Z-score for BMI	
Environment Rural or semi-rural home Mother smoked during pregnancy Second-hand smoke exposure Visible mold/dam	
Associated allergic disorders Family atopy in 1 parent or sibling History of food allergy History of allergic rhinitis Histo	
Asthma outcomes Age at first wheeze (months) No. of admissions for asthma exacerbation in childhood Asthma exacerb	
Allergic sensitization Atopy Total IgE (Ku/L) Blood eosinophil count (n/mm3)	

ACT: asthma control test, BD: bronchodilator, BMI: body mass index, FEV1: forced expiratory volume in one second, FVC: forced vital capacity, ICS: inhaled corticosteroids, IgE: type E immunoglobulin, LABA: long-acting beta-agonists, n': number of patients with data available (indicated in case of missing data), NSA: non-severe school-age asthmatic children, NSRW: non-severe preschool recurrent wheezers, PAQLQ: Pediatric Asthma Quality of Life Score, SA: severe school-age asthmatic children, SRW: severe preschool recurrent wheezers

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

	Total preschool population (N = 125)	**(a) NSRW (n = 47)**	**(b) SRW (n = 78)**	**p-value (a) vs (b)**	**Total school-age population (n = 170)**	**(c) NSA (n = 108)**	**(d) SA (n = 62)**	**p-valu (c) vs**
No sensitization	61 (49.6%)	17 (36.2%)	44 (57.9%)	0.019	42 (24.7%)	24 (22.2%)	18 (29%)	0.32
Number of positive c-sIgE determinants	6 [3-10]	6 [3.3-11.5]	5.5 [2.0-7.8]	0.44	7 [5-14]	7 [4.8-15]	9 [5-12]	0.48
Mono-sensitization (1 biological source)	19 (15.2%)	6 (12.8%)	13 (16.7%)	0.56	23 (13.5%)	6 (9.7%)	17 (15.7%)	0.27
Multi-sensitization ([?] 2 biological sources)	43 (34.4%)	24 (51.1%)	19 (24.4%)	0.0023	105 (61.8%)	67 (62%)	38 (61.3%)	0.92

	Total preschool population (N = 125)	**(a) NSRW (n = 47)**	**(b) SRW (n = 78)**	**p-value (a) vs (b)**	**Total school-age population (n = 170)**	**(c) NSA (n = 108)**	**(d) SA (n = 62)**	**p-value (c) vs**
Food biological source	26 (21.5%)	12 (25.5%)	14 (18.9%)	0.39 0.99	40 (23.7%)	25 (23.1%)	15 (24.6%)	0.83 0.059
egg	5 (4%)	2 (4.3%)	3 (3.8%)	0.99	5 (2.9%)	1 (0.9%)	4 (6.5%)	0.14
cow's milk	2 (1.6%)	1 (2.1%)	1 (1.3%)	0.54	4 (2.4%)	1 (0.9%)	3 (4.8%)	0.059
fish	1 (0.8%)	0 (0%)	1 (1.3%)	0.50	5 (2.9%)	1 (0.9%)	4 (6.5%)	0.67
shrimp	5 (4%)	2 (4.3%)	3 (3.8%)	0.99	6 (3.5%)	3 (2.8%)	3 (4.8%)	0.097
nuts	10 (8%)	5 (10.6%)	5 (6.4%)	0.99	21 (12.4%)	10 (9.3%)	11 (18%)	0.084
legumes	12 (10%)	5 (10.9%)	7 (9.5%)	0.71	16 (9.4%)	7 (6.5%)	9 (14.5%)	0.99
cereals	8 (6.4%)	2 (4.3%)	6 (7.7%)	0.99	3 (1.8%)	2 (1.9%)	1 (1.6%)	0.99
fruit	1 (0.8%)	0 (0%)	1 (1.3%)	0.63	11 (6.5%)	7 (6.5%)	4 (6.5%)	
Airborne biological source	56 (45.9%)	28 (60.9%)	26 (36.8%)	0.0098 0.14	126 (74.1%)	82 (75.9%)	44 (71%)	0.48 0.36
Polcalcin	2 (1.6%)	2 (4.3%)	0 (0%)	0.19	1 (0.6%)	0 (0%)	1 (1.6%)	0.98
Grass pollen	22 (17.6%)	11 (23.4%)	11 (14.1%)	0.065 0.20	66 (38.8%)	42 (38.9%)	24 (38.7%)	0.79 0.69
Tree pollen	15 (12.5%)	9 (19.6%)	6 (8.1%)	0.071	52 (30.8%)	34 (31.5%)	18 (29.5%)	0.76
Weed pollen	6 (4.8%)	4 (8.5%)	2 (2.6%)	0.99	25 (14.7%)	15 (13.9%)	10 (16.1%)	0.79
Animals	22 (17.6%)	12 (25.5%)	10 (12.8%)	0.0011 0.14	66 (38.8%)	41 (38%)	25 (40.3%)	0.47 0.99
Mold	9 (7.2%)	3 (6.4%)	6 (7.7%)	0.52 - -	15 (8.8%)	10 (9.3%)	5 (8.1%)	0.46 0.36 -
HDM	44 (35.2%)	25 (53.2%)	19 (24.4%)		102 (60%)	67 (62%)	35 (56.5%)	
Insects	2 (1.6%)	2 (4.3%)	0 (0%)		6 (3.5%)	4 (3.7%)	2 (3.2%)	
Venom	2 (1.7%)	0 (0%)	2 (2.7%)		8 (4.7%)	4 (3.7%)	4 (6.6%)	
Parasite	0 (0%)	0 (0%)	0 (0%)		1 (0.6%)	0 (0%)	1 (1.6%)	
Latex	0 (0%)	0 (0%)	0 (0%)		0 (0%)	0 (0%)	0 (0%)	

	Total preschool population (N = 125)	**(a) NSRW (n = 47)**	**(b) SRW (n = 78)**	**p-value (a) vs (b)**	**Total school-age population (n = 170)**	**(c) NSA (n = 108)**	**(d) SA (n = 62)**	**p-value (c) vs**
Cross-Reactive	25 (20%)	12 (25.5%)	13 (16.7%)	0.23 0.53	56 (32.9%)	34 (31.5%)	22 (35.5%)	0.59 0.10
Aller-gens	11 (8.8%) 5	3 (6.4%) 2 (4.3%)	8 (10.3%)	0.99 0.65	11 (6.5%) 6	4 (3.7%) 2 (1.9%)	7 (11.3%)	0.19 0.0046
Tropomyosin	(4%) 5	1 (2.1%)	3 (3.8%)	0.67	(3.5%)	4 (3.7%)	4 (6.5%)	0.66
Serum	(4%) 6	3 (6.4%)	4 (5.1%)	0.56	14	23	10	0.29
albumin	(4.8%) 3	2 (4.3%)	3 (3.8%)	0.14	(8.2%)	(21.3%)	(16.1%)	0.33
nsLTP	(2.4%) 2	2 (4.3%)	1 (1.3%)	0.36	38	4 (3.7%)	15	0.23
PR-10	(1.6%) 5	3 (6.4%)	0 (0%) 2		(22.4%)	9 (8.3%)	(24.2%)	
TLP	(4%)		(2.6%)		9 (5.3%)	6 (5.6%)	5 (8.1%)	
Profilin					11		2 (3.2%)	
CCD					(6.5%)		1 (7%)	
					13 (7.6%)			

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

	Cluster 1 Multiple, mainly grass pollens and PR-10 n = 4	**Cluster 2 Multiple, mainly food, grass pollens, animal dander and nsLTP N = 4**	**Cluster 3 Few, mainly HDM n = 53**	**p-value**
Severe recurrent wheeze	1 (25%)	3 (75%)	27 (51%)	0.46
Z-score FEV1	n'=2 -0.10 [-0.49, 0.29]	n'=3 - 0.59 [-0.65, -0.34]	n'=38 0.015 [-0.51, 0.86]	0.48
Z-score FEV1/FVC	-0.63 [-0.72, -0.53]	-1.36 [-1.5, -0.95]	0.010 [-1.13, 0.64]	0.32

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

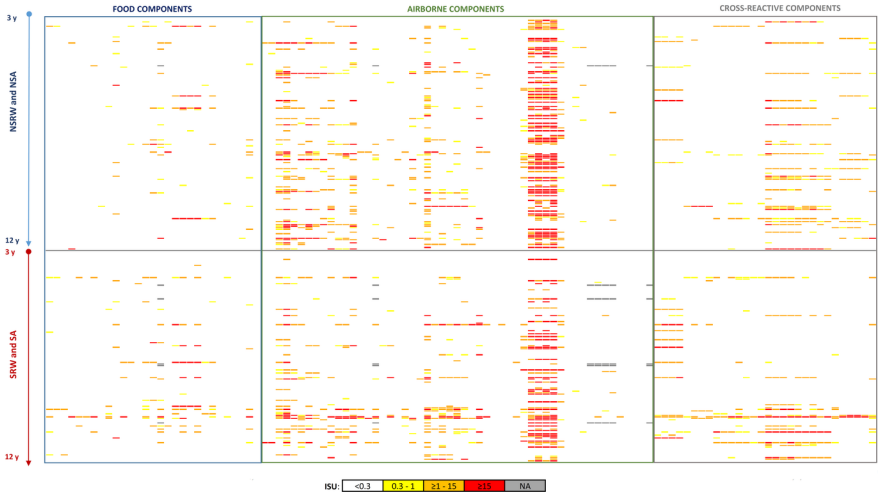
	Cluster 4 Multiple, mainly grass pollens, HDM, PR-10 and nsLTP n = 4	**Cluster 5 Multiple, mainly airborne including grass pollens and HDM n = 6**	**Cluster 6 Multiple, mainly grass pollens, HDM and PR-10 N = 24**	**Cluster 7 Few, mainly HDM n = 94**	**p-value**
Severe asthma	4 (100%)	2 (33%)	6 (25%)	32 (34%)	0.036
Z-score FEV1	n'=4 -0.89 [-1.92, 0.078]	n'=6 0.6 [0.35, 0.92]	n'=23 0.08 [-0.87, 0.93]	n'=89 -0.34 [-1.16, 0.42]	0.27
Z-score FEV1/FCV	-1.69 [-2.1, -1.4]	-0.92 [-1.33, -0.25]	-0.44 [-1.37, 0.11]	-0.67 [-1.48, 0.18]	0.25

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

ISAC: ImmunoCAP Immuno Solid-phase Allergen Chip, NSA: non-severe school-age asthmatic children, NSRW: non-severe preschool recurrent wheezers, SA: severe school-age asthmatic children, SRW: severe preschool recurrent wheezers

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



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