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COBRAPed cohort: Do sensitization patterns 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 determine whether sensitization patterns can discriminate between children from the French COBRAPed cohort with NSRW/NSA and those with SRW/SA.

Methods: IgE to 112 components (c-sIgE) (ImmunoCAP® ISAC) were analyzed in 125 preschools (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%, $p < .01$). Sensitization to non-specific lipid transfer protein (nsLTP) components was more frequent among SA than NSA (16%

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 1 s; 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.

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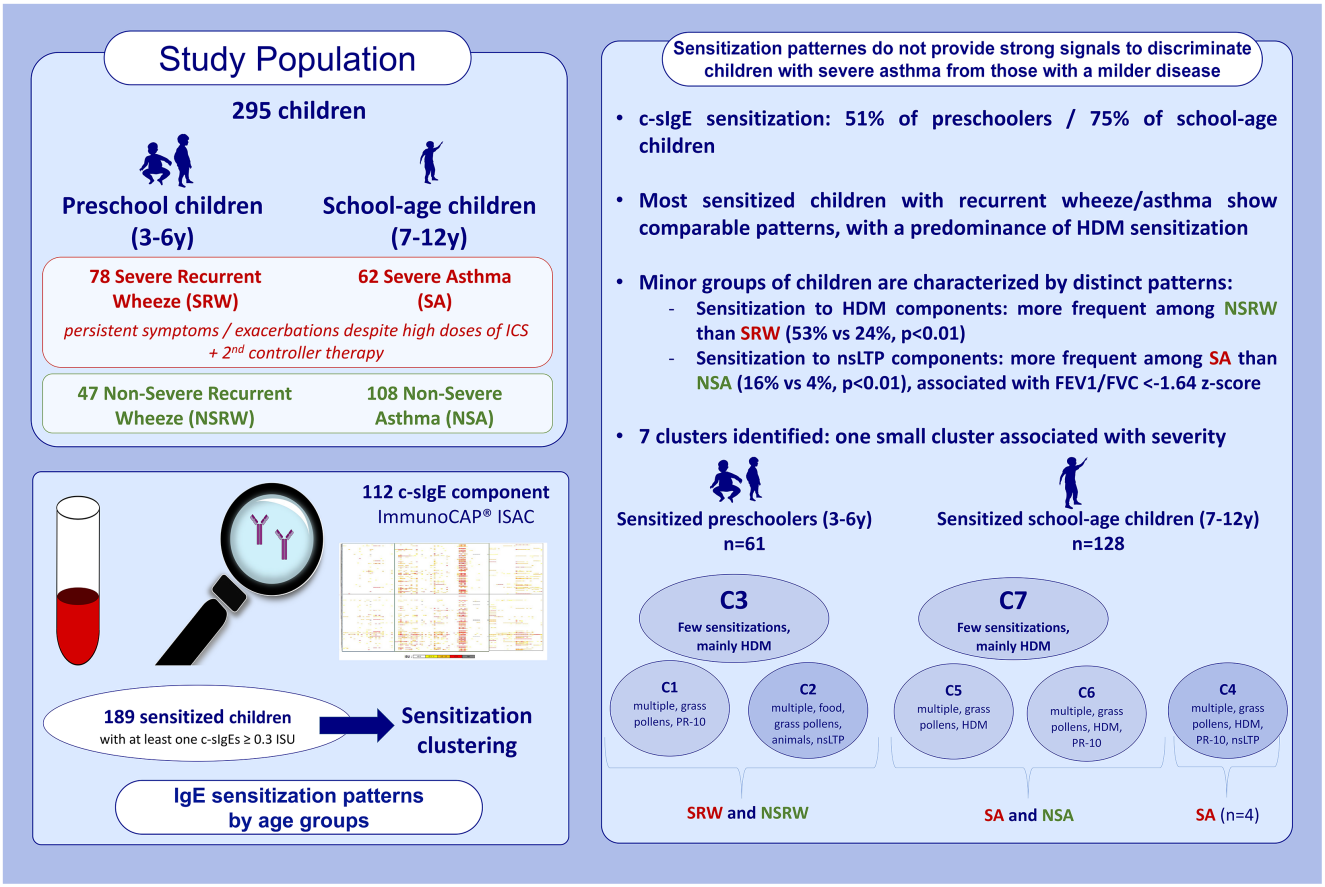
vs. 4%, $p < .01$) and associated with an FEV1/FVC < −1.64 *z*-score. Among sensitized children, seven clusters with varying patterns were identified. The two broader clusters identified in each age group were characterized by "few sensitizations, mainly to HDM." One cluster ($n = 4$) with "multiple 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, sensitization patterns did not provide strong signals to 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.

KEYWORDS
asthma, preschool, school-age, sensitization, severe asthma



GRAPHICAL ABSTRACT

The contents of this page will be used as part of the graphical abstract of html only. It will not be published as part of main article.
IgE sensitization patterns in severe recurrent wheeze/school-age asthma.

1 | INTRODUCTION

Severe asthma (SA) in school-age children (7–12 years) and severe recurrent wheeze (SRW) in preschoolers (3–6 years) are characterized by multiple phenotypes.[1–4]

Early and multiple sensitizations are associated with severe persistent asthma and lung function (LF) 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

Key message

Children with wheeze/asthma display frequent occurrences and high levels of sensitization, but c-sIgE sensitization patterns did not provide strong signals to 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.

individual allergen molecules (components, c-sIgE) and has been used to characterize sensitization profiles in children.[5–7,10] Previous results from the Pediatric Cohort of Bronchial Obstruction and Asthma (COBRAPed) of preschool and school-age children with recurrent wheeze/asthma suggest a role for both environmental factors and atopy in asthma severity.[11] The description of sensitization profiles using CRD provides an opportunity to further study the relationship between allergic sensitization and asthma severity during childhood. We aimed to determine whether sensitization patterns can discriminate between children with SA/SRW and those with milder disease.

2 | METHODS

2.1 | Study design and participants

A description of the cohort has been published[11,12] and is available in the Online Supplement. Ethical approval and written informed consent were obtained. The study is registered in ClinicalTrial.gov (NCT02114034).

Children were assigned to four groups: non-severe preschool recurrent wheezers (NSRW), severe preschool recurrent wheezers (SRW), non-severe school-age asthmatic children (NSA), and severe school-age asthmatic children (SA). Atopy was defined as having at least one positive skin-prick test and/or specific IgE levels (≥0.35 kuA/L) against airborne and/or food allergens. Patients with SRW and SA receiving omalizumab were excluded from this analysis.

2.2 | 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). 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) (Figure 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.).

2.3 | Statistical analysis

R V3.3.1 was used for statistical analysis. 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. Multivariable logistic regression analyses were built with the inclusion of all biological sources with univariate *p*-values <0.1. Resulting odds ratios (OR) were reported with a 95% confidence interval (CI)

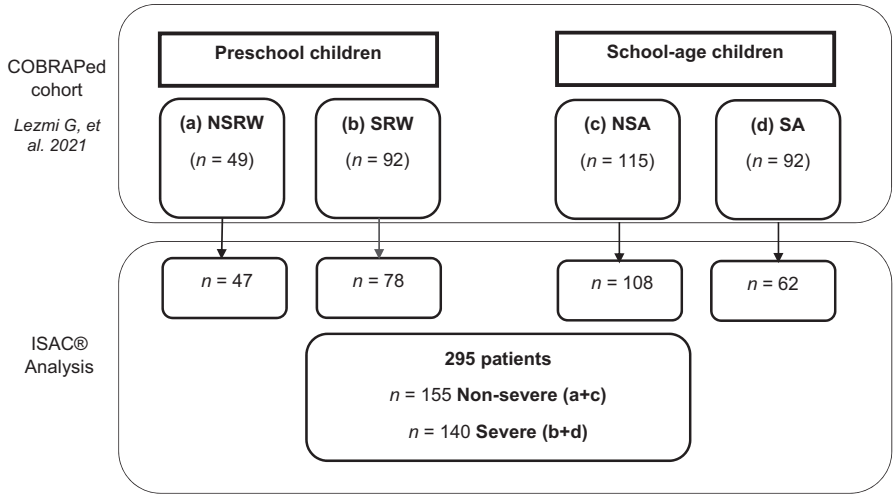


FIGURE 1 Flowchart of the study population. ISAC, immunoCAP immuno solid-phase allergen chip; NSA, non-severe school-age asthmatic children; NSRW, non-severe preschool recurrent wheezers; SA, severe school-age asthmatic children; SRW, severe preschool recurrent wheezers.

and *p*-values from the Wald Test. 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 as a graphical representation of data using a grid of colors (according to c-sIgE ISU level), with rows standing for individuals and columns 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 (Figure S1). 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. Area under the curve (AUC) values indicated the level of precision. Values below 0.60 were considered as failures. Prediction errors of the random forest analyses were calculated using out-of-bag 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] A Poisson prior distribution was applied for the number of clusters and a uniform distribution for the Bernoulli parameters.

3 | RESULTS

3.1 | Description of the population

Among the 295 children with available ISAC data, 47 were classified as NSRW, 78 as SRW, 108 as NSA, and 62 as SA (Figure 1). Their main characteristics are presented in Table 1. Briefly, children with SRW were more frequently exposed to second-hand smoke and visible mold/dampness. Children with SA had a more frequent history of food allergy and atopic dermatitis than those with NSA. Atopy status was similar between NSRW and SRW or NSA and SA, respectively.

3.2 | Sensitization profile differences between non-severe and severe patients

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, and 45.9% to at least one airborne allergen (Table 2). Preschool children with NSRW more frequently had multi-sensitization (≥2 biological sources) than those with SRW (51.1% vs. 24.4%, $p=.002$). Airborne allergen and house dust mite (HDM) sensitizations were more frequent among children with NSRW than SRW (60.9% vs. 36.8%; $p=.010$) and (53.2% vs. 24.4%; $p=.001$), respectively. HDM sensitization remained the only significant variable in multivariable regression analysis with an OR, (CI) of 0.28 (0.12–0.66) (Table S1). At the c-sIgE level, patterns of sensitization to individual allergen components did not discriminate NSRW from SRW or NSA from SA (Table 2, Figure 2). However, sensitization to the HDM components Der f 1 (38.3% vs. 26.4%, $p=.032$), Der f 2 (42.6% vs. 16.7%, $p=.003$), Der p 1 (42.6% vs. 19.2%, $p=.009$), and Der p 2 (48.9% vs. 17.9%, $p<.001$) was more frequent among children with NSA than SA (Table S2). There was no difference in terms of c-sIgE components ≥15 ISU (Table S3).

Among school-age children, at the biological source level, 23.7% were sensitized to at least one food, and 74.1% to at least one airborne (Table 2). The rates of multi-sensitization were comparable between children with NSA and SA (62% vs. 61.3%, $p=.92$). There was no difference in airborne sensitization profiles. At the c-sIgE level, sensitization to non-specific lipid transfer protein (nsLTP) was more frequent among children with SA than NSA (16.1% vs. 3.7%, $p=.005$), including the nsLTP components Art v 3 (8.1% vs. 0.9%, $p=.046$) and Cor a 8 (6.5% vs. 0, $p=.032$) (Table S2). Other sensitizations were more frequent among children with SA including sensitizations to the food components Gal d 1 (6.5% vs. 0%, $p=.032$) and Cor a 9 (8.1% vs. 0.9%, $p=.046$), the airborne components Can f 1 (22.6% vs. 8.3%, $p=.017$), Can f 2 (14.5% vs. 2.8%, $p=.01$). In the multivariable analysis, no significant effect was observed: OR (CI): egg, 2.9 (0.25–35); fish, 4.1 (0.35–47); nuts, 1.22 (0.40–3.7); legumes, 0.91 (0.23–3.7); nsLTP, 3.1 (0.71–13) (Table S1). The number of children with c-sIgE ≥15 ISU was comparable between SA and NSA (Table S3).

3.3 | 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 (Figure 2). There was an increase in the number of positive biological sources for airborne (RR 1.14 [1.08–1.20], $p<.0001$) and cross-reactive c-sIgE (RR 1.18 [1.07–1.30] per one-year increase, $p=.00098$), but not for food biological sources (Figure S2, Table S4).

3.4 | Lung function and sensitization profiles

Among the 235 participants with available data on LF, there was no relationship between c-sIgE sensitization and the forced expiratory volume in 1 s (FEV1)/forced vital capacity (FVC) *z*-score, except for the frequency of nsLTP sensitization, higher for the participants with a FEV1/FVC *z*-score <-1.64 than in the others (16.7% vs. 5.2%, $p=.017$) (Table S5).

TABLE 1 Description of the study population.

	Total preschool population ($N = 125$)	(a) NSRW ($n = 47$)	(b) SRW ($n = 78$)	p-value (a) versus (b)	Total school-age population ($n = 198$)	(c) NSA ($n = 108$)	(d) SA ($n = 62$)	p-value (c) versus (d)
Demographics								
Age, years	5 [4, 6]	5 [4, 6]	5 [4, 6]	.23	9 [8, 11]	9 [8.0, 10.2]	9 [9, 11]	.12
Male gender	84/125 (67.2)	32/47 (68.1)	52/78 (66.7)	.99	112/170 (65.9)	69/108 (63.9)	43/62 (69.4)	.58
Caucasian	91/119 (76.5)	35/42 (83.3)	56/77 (72.7)	.99	104/158 (65.8)	70/102 (68.6)	34/56 (60.7)	.41
Birth weight, g	3185 [2800, 3502]	3380 [3102, 3550]	3100 [2600, 3430]	.0036	3270 [2925, 3665]	3305 [2900, 3715]	3200 [2930, 3540]	.27
Z-score for BMI	0.3 [−0.5, 1.1]	0.3 [−0.3, 0.8]	0.4 [−0.6, 1.3]	.48	0.7 [−0.3, 2.0]	0.9 [−0.3, 2.0]	0.5 [−0.4, 1.7]	.32
	$n' = 124$	$n' = 47$	$n' = 77$		$n' = 163$	$n' = 102$	$n' = 61$	
Environment								
Rural or semi-rural home	56/125 (44.8)	23/47 (48.9)	33/78 (42.3)	.59	78/169 (46.2)	47/107 (43.9)	31/62 (50)	.55
Mother smoked during pregnancy	17/125 (13.6)	4/47 (8.5)	13/78 (16.7)	.31	18/168 (10.7)	10/106 (9.4)	8/62 (12.9)	.66
Second-hand smoke exposure	18/122 (14.8)	1/47 (2.1)	17/75 (22.7)	.0044	8/164 (4.9)	5/104 (4.8)	3/60 (5.0)	.99
Visible mold/dampness at home	26/124 (21.0)	4/46 (8.7)	22/78 (28.2)	.019	28/168 (16.7)	16/106 (15.1)	12/62 (19.4)	.62
Pet ownership	57/125 (45.6)	19/47 (40.4)	38/78 (48.7)	.47	95/169 (56.2)	58/107 (54.2)	37/62 (59.7)	.60
Associated allergic disorders								
Family atopy in 1 parent or sibling	102/120 (85.0)	40/46 (87.0)	62/74 (83.8)	.47	138/160 (86.2)	87/102 (85.3)	51/58 (87.9)	.82
History of food allergy	18/120 (15.0)	7/46 (15.2)	11/74 (14.9)	.99	30/166 (18.1)	14/106 (13.2)	16/60 (26.7)	.051
History of allergic rhinitis	61/124 (49.2)	27/46 (58.7)	34/78 (43.6)	.15	127/170 (74.7)	79/108 (73.1)	48/62 (77.4)	.66
History of atopic dermatitis	42/125 (33.6)	16/47 (34.0)	26/78 (33.3)	.99	58/170 (34.1)	29/108 (26.9)	29/62 (46.8)	.014
Asthma outcomes								
Age at first wheeze (months)	5 [2, 8] $n' = 104$	6 [3.8, 8.0] $n' = 36$	4 [2.0, 8.2] $n' = 68$	/	5 [2, 9] $n' = 120$	6 [2, 11] $n' = 73$	4 [2.0, 8.5] $n' = 47$	/
No. of admissions for asthma exacerbation in childhood	1 [0, 4]	0 [0, 1]	3 [0, 5]	/	0 [0, 2]	0 [0, 1]	2 [0, 5]	/
Asthma exacerbation in the previous year	$n' = 125$	$n' = 47$	$n' = 78$		$n' = 168$	$n' = 107$	$n' = 61$	
ACT score	71/123 (57.7)	3/47 (6.4)	68/76 (89.5)	/	53/169 (31.4)	8/107 (7.5)	45/62 (72.6)	/
Total PAQLQ score	20 [16, 23] $n' = 119$	23 [22, 25] $n' = 43$	18 [14, 21] $n' = 76$	/	21 [17, 24] $n' = 170$	23 [20.0, 24.2] $n' = 108$	17 [13.2, 20.0] $n' = 62$	/
FEV1 pre-BD (*Z*-score)	–	–	–		6 [4.8, 6.8] $n' = 167$	6.2 [5.4, 6.8] $n' = 105$	5.2 [3.7, 6.4] $n' = 62$	/
FEV1/FVC pre-BD (*Z*-score)	−0.1 [−0.9, 0.9] $n' = 77$	0 [−0.5, 0.9] $n' = 37$	−0.3 [−1.3, 0.9] $n' = 40$	/	−0.1 [−1.2, 0.6] $n' = 160$	0.1 [−0.8, 0.8] $n' = 103$	−0.7 [−1.9, 0.2] $n' = 57$	/

(Continues)

TABLE 1 (Continued)

	Total preschool population ($N=125$)	(a) NSRW ($n=47$)	(b) SRW ($n=78$)	p-value (a) versus (b)	Total school-age population ($n=198$)	(c) NSA ($n=108$)	(d) SA ($n=62$)	p-value (c) versus (d)
ICS	−0.1 [−1.2, 0.7] $n'=77$	−0.1 [−0.9, 0.4] $n'=37$	−0.2 [−1.4, 1.1] $n'=40$	/	−0.7 [−1.5, 0.1] $n'=158$	−0.6 [−1.2, 0.1] $n'=101$	−1.5 [−2.4, −0.1] $n'=57$	/
ICS doses (µg/d eq Budesonide)	115/125 (92.0)	37/47 (78.7)	78/78 (100.0)	/	136/170 (80.0)	74/108 (68.5)	62/62 (100.0)	/
	800 [400, 1600]	400 [400, 400]	1000 [800, 1837]	/	500 [400, 1000]	400 [250, 500]	1000 [800, 1150]	/
ICS + LABA	$n'=115$	$n'=37$	$n'=78$		$n'=136$	$n'=74$	$n'=62$	
	48/124 (38.7)	13/46 (27.7)	35/77 (45.5)	/	101/170 (59.4)	48/108 (44.4)	53/62 (85.5)	/
Allergic sensitization								
Atopy	65/106 (61.3)	30/43 (69.8)	35/63 (55.6)	.20	125/152 (82.2)	78/97 (80.4)	47/55 (85.5)	.57
Total IgE (Ku/L)	89 [28.2, 317.2]	129 [41.8, 428.2]	84.5 [25.2, 235.8]	.24	283 [87.5, 691.5]	300.5 [94.0, 676.8]	239 [74.0, 811.5]	.70
	$n=114$	$n'=40$	$n'=74$		$n'=159$	$n'=100$	$n'=59$	
Blood eosinophil count (n/mm^3)	250 [120, 500]	300 [135, 600]	240 [105, 475]	.40	400 [200, 700]	320 [200, 600]	400 [195.5, 800.0]	.41
	$n=101$	$n'=39$	$n'=62$		$n'=124$	$n'=77$	$n'=47$	

Note: For asthma outcomes, differences between groups reflect inclusion criteria, thus p values are not mentioned. Continuous variables are presented as medians [interquartile range] and categorical variables as numbers (%).

Abbreviations: 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 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) versus (b)	Total school-age population ($n = 170$)	(c) NSA ($n = 108$)	(d) SA ($n = 62$)	p-value (c) versus (d)
No sensitization	61 (49.6%)	17 (36.2%)	44 (57.9%)	.019	42 (24.7%)	24 (22.2%)	18 (29%)	.32
Number of positive c-sIgE determinants	6 [3–10]	6 [3.3–11.5]	5.5 [2.0–7.8]	.44	7 [5–14]	7 [4.8–15]	9 [5–12]	.48
Mono-sensitization (1 biological source)	19 (15.2%)	6 (12.8%)	13 (16.7%)	.56	23 (13.5%)	6 (9.7%)	17 (15.7%)	.27
Multi-sensitization (≥2 biological sources)	43 (34.4%)	24 (51.1%)	19 (24.4%)	.0023	105 (61.8%)	67 (62%)	38 (61.3%)	.92
Food biological source	26 (21.5%)	12 (25.5%)	14 (18.9%)	.39	40 (23.7%)	25 (23.1%)	15 (24.6%)	.83
Egg	5 (4%)	2 (4.3%)	3 (3.8%)	.99	5 (2.9%)	1 (0.9%)	4 (6.5%)	.059
Cow's milk	2 (1.6%)	1 (2.1%)	1 (1.3%)	.99	4 (2.4%)	1 (0.9%)	3 (4.8%)	.14
Fish	1 (0.8%)	0 (0%)	1 (1.3%)	.54	5 (2.9%)	1 (0.9%)	4 (6.5%)	.059
Shrimp	10 (8%)	5 (10.6%)	5 (6.4%)	.50	5 (2.9%)	3 (2.8%)	3 (4.8%)	.67
Nuts	12 (10%)	5 (10.9%)	7 (9.5%)	.99	6 (3.5%)	10 (9.3%)	11 (18%)	.097
Legumes	8 (6.4%)	2 (4.3%)	6 (7.7%)	.71	21 (12.4%)	7 (6.5%)	9 (14.5%)	.084
Cereals	1 (0.8%)	0 (0%)	1 (1.3%)	.99	16 (9.4%)	2 (1.9%)	1 (1.6%)	.99
Fruit	4 (3.2%)	2 (4.3%)	2 (2.6%)	.63	3 (1.8%)	7 (6.5%)	4 (6.5%)	.99
Airborne biological source	56 (45.9%)	28 (60.9%)	26 (36.8%)	.0098	126 (74.1%)	82 (75.9%)	44 (71%)	.48
Polcalcin	2 (1.6%)	2 (4.3%)	0 (0%)	.14	1 (0.6%)	0 (0%)	1 (1.6%)	.36
Grass pollen	22 (17.6%)	11 (23.4%)	11 (14.1%)	.19	66 (38.8%)	42 (38.9%)	24 (38.7%)	.98
Tree pollen	15 (12.5%)	9 (19.6%)	6 (8.1%)	.065	52 (30.8%)	34 (31.5%)	18 (29.5%)	.79
Weed pollen	6 (4.8%)	4 (8.5%)	2 (2.6%)	.20	25 (14.7%)	15 (13.9%)	10 (16.1%)	.69
Animals	22 (17.6%)	12 (25.5%)	10 (12.8%)	.071	66 (38.8%)	41 (38%)	25 (40.3%)	.76
Mold	9 (7.2%)	3 (6.4%)	6 (7.7%)	.99	15 (8.8%)	10 (9.3%)	5 (8.1%)	.79
HDM	44 (35.2%)	25 (53.2%)	19 (24.4%)	.0011	102 (60%)	67 (62%)	35 (56.5%)	.47
Insects	2 (1.6%)	2 (4.3%)	0 (0%)	.14	6 (3.5%)	4 (3.7%)	2 (3.2%)	.99
Venom	2 (1.7%)	0 (0%)	2 (2.7%)	.52	8 (4.7%)	4 (3.7%)	4 (6.6%)	.46
Parasite	0 (0%)	0 (0%)	0 (0%)	–	1 (0.6%)	0 (0%)	1 (1.6%)	.36
Latex	0 (0%)	0 (0%)	0 (0%)	–	0 (0%)	0 (0%)	0 (0%)	–
Cross-reactive allergens	25 (20%)	12 (25.5%)	13 (16.7%)	.23	56 (32.9%)	34 (31.5%)	22 (35.5%)	.59
Tropomyosin	11 (8.8%)	3 (6.4%)	8 (10.3%)	.53	11 (6.5%)	4 (3.7%)	7 (11.3%)	.10
Serum albumin	5 (4%)	2 (4.3%)	3 (3.8%)	.99	6 (3.5%)	2 (1.9%)	4 (6.5%)	.19
nsLTP	5 (4%)	1 (2.1%)	4 (5.1%)	.65	14 (8.2%)	4 (3.7%)	10 (16.1%)	.0046

(Continues)

TABLE 2 (Continued)

	Total preschool population ($N=125$)	(a) NSRW ($n=47$)	(b) SRW ($n=78$)	p-value (a) versus (b)	Total school-age population ($n=170$)	(c) NSA ($n=108$)	(d) SA ($n=62$)	p-value (c) versus (d)
PR-10	6 (4.8%)	3 (6.4%)	3 (3.8%)	.67	38 (22.4%)	23 (21.3%)	15 (24.2%)	.66
TLP	3 (2.4%)	2 (4.3%)	1 (1.3%)	.56	9 (5.3%)	4 (3.7%)	5 (8.1%)	.29
Profilin	2 (1.6%)	2 (4.3%)	0 (0%)	.14	11 (6.5%)	9 (8.3%)	2 (3.2%)	.33
CCD	5 (4%)	3 (6.4%)	2 (2.6%)	.36	13 (7.6%)	6 (5.6%)	1 (7%)	.23

Note: c-sIgE were dichotomized using a binary threshold (positive ≥0.30 ISU).

Abbreviations: 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.

3.5 | Supervised multivariate 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. Similarly, the random forest 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 (Figure S3).

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, the random forest 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 (Figure S4).

3.6 | 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) 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" (Figure S5). The distribution of SRW within the three clusters did not differ, but three of the four patients of Cluster 2 had SRW. LF parameters were similar between the 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" (Figure S6). All four patients from Cluster 4 had SA, versus 33% in Cluster 5, 25% in Cluster 6, and 34% in Cluster 7 ($p=.036$). LF parameters were comparable between the clusters (Tables 4 and S6).

4 | DISCUSSION

4.1 | Main results

We aimed to determine whether sensitization profiles of children with SRW or SA could be distinguishable from those with NSRW or NSA using a CRD multiplex assay. Overall, the patterns of biological source sensitization did not discriminate between children with NSRW and SRW or with NSA and SA. At the c-sIgE level, sensitization to airborne allergens, especially towards HDM components, and multi-sensitization, were approximately twice as frequent among preschoolers with NSRW than with SRW. At school age, sensitization to Gal d 1, hazelnut 2S globulin, dog salivary lipocalin proteins,

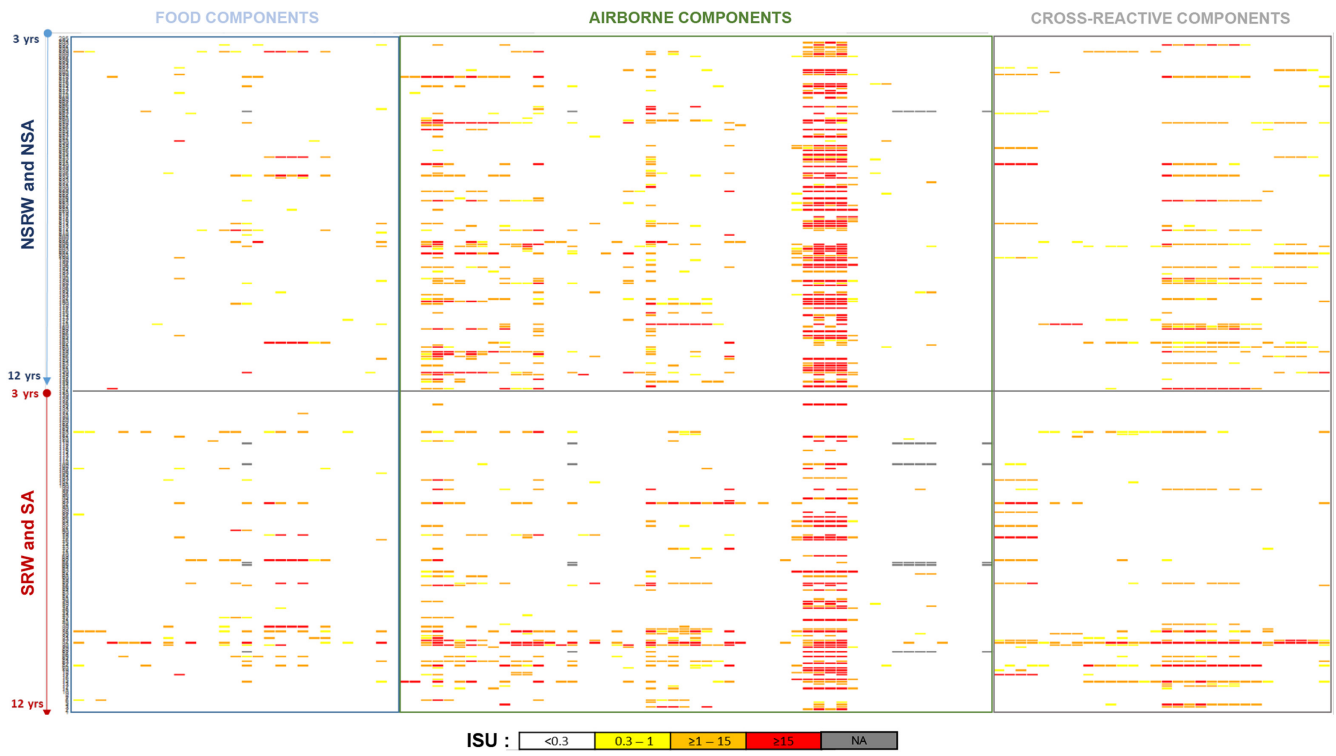


FIGURE 2 Patterns of sensitization to each allergen component (columns) for individual participants (rows) stratified by severity group. ISU, ISAC Standardized Units; NSA, non-severe school-age asthmatic children; NSRW, non-severe preschool recurrent wheezers; SA, severe school-age asthmatic children; SRW, severe preschool recurrent wheezers; yrs, years.

TABLE 3 Severity and LF 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%)	.46
	$n'=2$	$n'=3$	$n'=38$	
Z-score FEV1	−0.10 [−0.49, 0.29]	−0.59 [−0.65, −0.34]	0.015 [−0.51, 0.86]	.48
Z-score FEV1/FVC	−0.63 [−0.72, −0.53]	−1.36 [−1.5, −0.95]	0.010 [−1.13, 0.64]	.32

Abbreviations: FEV1, forced expiratory volume in 1 s; FVC, forced vital capacity; HDM, house dust mite; PR-10, pathogenesis-related protein family 10.

and nsLTP was more frequent among children with SA, and sensitization to nsLTP was associated with impaired LF. Unsupervised clustering confirmed the heterogeneity in sensitization profiles, identifying three clusters for preschoolers and four for school-age children with shared patterns but also some specificities (grass and PR-10 among preschoolers and nsLTP among school-age children). Only one small cluster with multiple airborne and nsLTP sensitization was associated with asthma severity at school age.

4.2 | Most sensitized children with recurrent wheezing/asthma show comparable patterns

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 described in the U-BIOPRED cohort.[10] Sensitization to HDM and multi-sensitization were even more frequent among preschoolers with NSRW than those with SRW, supporting that disease severity is associated with exposure to mold and cigarette smoke rather than atopy in this age group.[11] This suggests that the drivers of inflammation may differ between NSRW and SRW. In this regard, in a previous paper by our teams, airway inflammation in SRW was found to be more neutrophilic than eosinophilic.[16] One could hypothesize that the imbalance between type 2 and non-type 2 mechanisms in the preschool years favors a more severe presentation in SRW. The finding that patterns of sensitization to biological sources did not discriminate between children with SA/SRW and those with milder disease confirms the results from the U-BIOPRED cohort.[10] The similarity of sensitization profiles between children from the two groups suggests that, at least among sensitized children, asthma in school-age children may share common features with wheezing in preschoolers.

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%)	.036
	$n'=4$	$n'=6$	$n'=23$	$n'=89$	
Z-score FEV1	−0.89 [−1.92, 0.078]	0.6 [0.35, 0.92]	0.08 [−0.87, 0.93]	−0.34 [−1.16, 0.42]	.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]	.25

Abbreviations: FEV1, forced expiratory volume in 1 s; FVC, forced vital capacity; HDM, house dust mite; PR-10, pathogenesis-related protein family 10.

These results confirm that sensitization patterns may not be useful biomarkers of disease severity in children when described in terms of numbers/levels of c-sIgE sensitization at a single time point.[10] More complex endotypic mechanisms than simple allergenic sensitization may underpin asthma severity during childhood.

4.3 | Sensitization to certain single components is associated with severity, in particular to nsLTP

Sensitization to Gal d 1 and Cor a 9 and Can f 1 and Can f 2 was more frequent among children with SA than those with NSA. These results confirm that Can f 2 sensitization and multi-sensitization to lipocalins are more frequent among children with SA than those with milder disease.[17,18] 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 LF. 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.[19–21] Sensitization toward nsLTP from pollen and food was observed. It is yet to be determined whether sensitization to nsLTP primarily occurs through pollen or food exposure.[20] Although these results need confirmation, they highlight how geographical variation might affect asthma severity.

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

We observed an increase in sensitization between the ages 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 LF impairment.[5–9] In contrast with other studies, we did not observe any relationship between mold sensitization and SRW/SA.[22–24] However, mold sensitization was retained in only a limited number of children in our study 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. An unbalanced immune reaction biased toward a response involving type 2 helper T cells may be involved in children with early and multiple sensitizations[9,25] and exacerbated interferon production in response to viral infections in children with late-onset sensitization and asthma.[25]

4.5 | Strengths and limitations

The 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.[10] This study had several limitations. We excluded patients receiving omalizumab for obvious reasons, thus severe and often highly atopic patients were excluded.[26,27] However, omalizumab being mostly offered to school-age children, did not influence results for the preschoolers. Because our analysis was exploratory, with no a priori hypothesis, we have not corrected the p-values for multiple testing, which can be seen as a limitation of our study. If we had applied this correction, it would have probably shown null results, further reinforcing our conclusion that overall, sensitization patterns may not be useful biomarkers of disease severity in children and that the severity of asthma may rely on more complex mechanisms than sensitization.

5 | CONCLUSION

Sensitization was frequent in our cohort even among preschoolers. However, sensitization patterns did not provide strong signals to discriminate children with severe disease from those with milder disease, suggesting that other mechanisms underpin asthma severity.

AUTHOR CONTRIBUTIONS

Stéphanie Lejeune: Formal analysis (equal); methodology (equal); writing – original draft (equal); writing – review and editing (equal). **Naïm Bouazza:** Data curation (equal); formal analysis (equal); methodology (equal); validation (equal); writing – original draft (equal); writing

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CONFLICT OF INTEREST STATEMENT

In the past 3 years, SL declares having received research grants from Astra Zeneca and Santelys, and 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, and 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.

PEER REVIEW

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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