

Extract and component-specific sensitization patterns in Canadian moderate-to-severe preschool asthmatics

To the Editor:

Preschool children with severe asthma are challenging in management. This relates to the lack of objective assessments available for this age-group that can help distinguish between children with transient wheezing and those with persistent wheezing syndromes that develop into moderate-to-severe asthma phenotypes.¹ Within this clinically heterogeneous group, the presence of allergic sensitization is considerable. Despite being an established risk factor for asthma development, information is limited regarding the degree of atopic predisposition, diversity of allergic sensitization, and impact of environmental allergens as potential trigger factors for this challenging group of wheezing preschool children in eastern North America.

This descriptive study reports baseline allergic sensitization patterns observed within a well-characterized high-risk Canadian

preschool cohort with moderate-to-severe asthma. Sensitization profiles were obtained in 87 children. Patients between 3 and 6 years of age were prospectively recruited based on a presentation of acute wheezing at the emergency department (ED) at the Hospital for Sick Children (Toronto, Canada) and a history of least 2 prior episodes of wheezing in the past year (12 months). Skin prick testing (SPT) to 14 allergen extracts (ALK-Abelló) (Table S1) and specific IgE measurements to 156 allergen extracts and 126 allergen components (Allergy Explorer, Macro Array Dx, Austria) were conducted in a representative subgroup with serum samples available (Table S2). A total of 21 allergen sources and 66 corresponding allergen components were considered for analysis (Table S1). A positive allergic sensitization was defined as either a positive SPT (≥ 2 mm) or a positive sIgE (≥ 0.30 kUa/L) measurement to the allergen extract or any corresponding component.

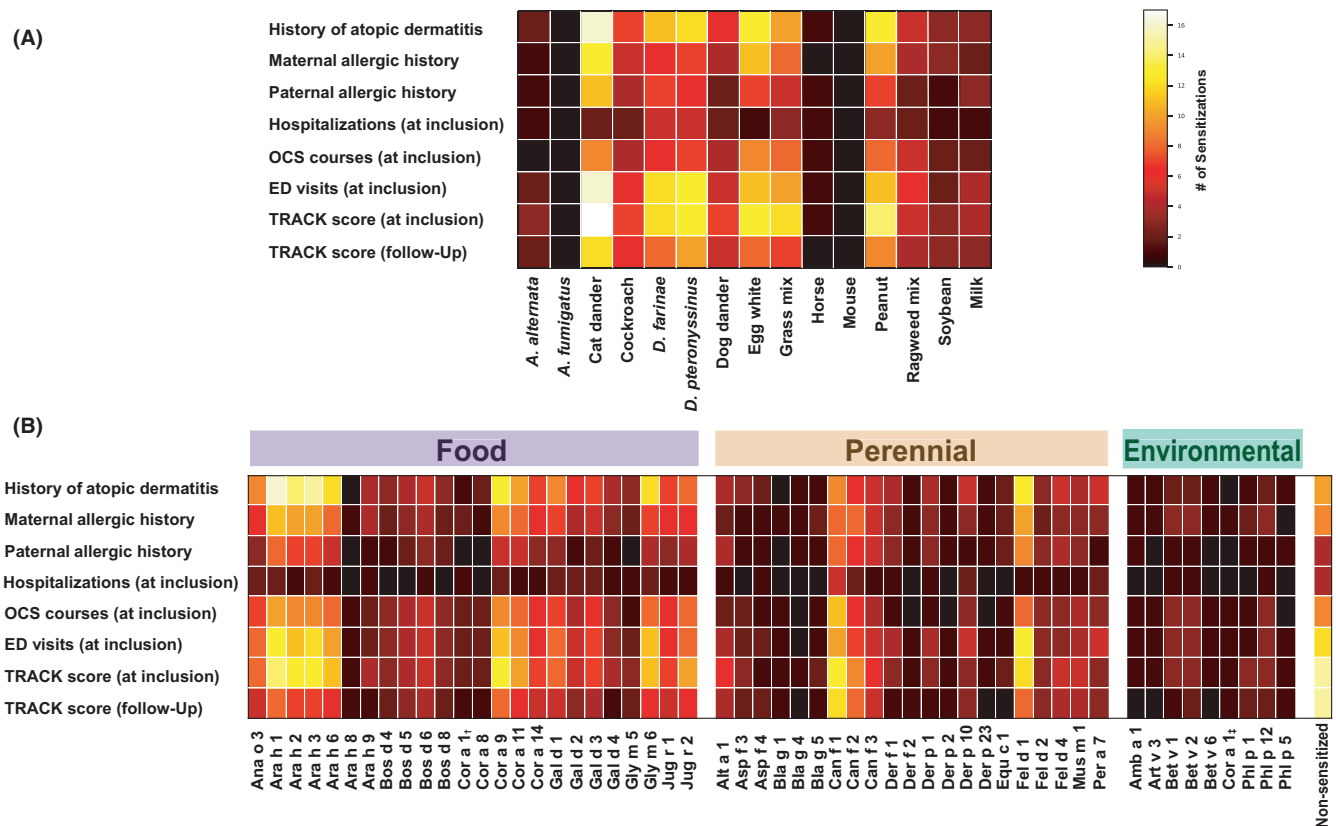


FIGURE 1 Concurrence between atopic history and clinical asthma markers with positive sensitizations against (A) Skin prick testing to allergen extracts from full cohort (N = 87) and (B) sIgE to allergen components from a subgroup of 55 patients. Scale indicates number of cases with concurrent phenotype and sensitization

Abbreviations: AD, atopic dermatitis; Ara h 1, 2, 3, 6, 8, 9, peanut Can f 1 dog allergen; CAPS, Childhood Asthma Prevention Study; CHILD study, Canadian Healthy Infant Longitudinal Development Study; Cor a 9, 11, 14, hazelnut allergen; ED, emergency department; Fel d 1, cat allergen; Gly m 6, soy allergen; ICS, inhaled corticosteroid; LISaplus, influence of Lifestyle-related factors on the development of the Immune System and Allergies in East and West Germany study; MACS, Melbourne Atopic Cohort Study; OCS, oral corticosteroid; sIgE, allergen-specific IgE; TRACK, Test for Respiratory and Asthma Control in Kids.

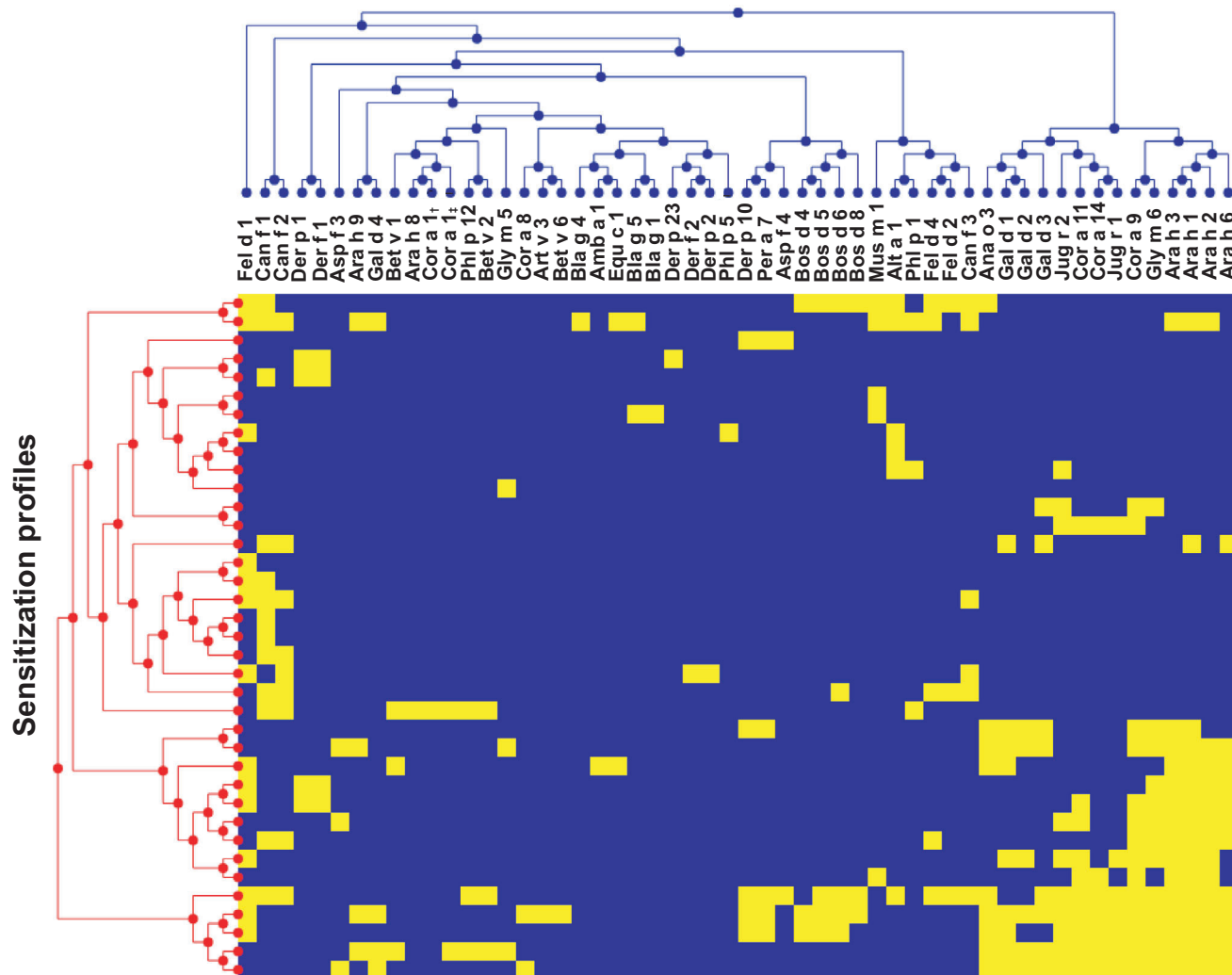


FIGURE 2 Hierarchical clustering performed on patient sensitization profiles and allergen components indicating clusters by allergen source and biochemical family (sensitized: N = 37; nonsensitized: N = 18). Yellow indicates positive sensitization to allergen component (sIgE $\geq$ 0.3 kUa/L); blue indicates negative sensitization

Sixty-seven percent of children were sensitized to any of the allergenic sources, with the majority being oligo-/polysensitized (60%) (Table S2). Sensitizations occurred most frequently due to the following sources: food allergens, predominantly peanut (35%), soy (25%), egg (24%), tree nuts (20%–29%), and animal/dander allergens, primarily cat (29%) and dog (25%) (Figure S1). Thus, prevalence of allergic sensitizations in this asthmatic preschool cohort is highly elevated as compared to an unselected Canadian pediatric population (Canadian Healthy Infant Longitudinal Development study [CHILD]) reporting that 14.4% of 3-year-old children were sensitized and 6.3% polysensitized.² Compared to this study, the age-matched CHILD study reported sensitization only by SPT; however, the major allergen sources were consistent between these two Canadian cohorts. Cat (5.1%) and peanut (4.0%) were the most frequent sensitizations at this age in the CHILD study.² The same set of SPT solutions was used in both studies.

Peanut and cat sensitization displayed the strongest association with the clinical surrogate markers of uncontrolled asthma, defined

by an exacerbation frequency with at least 2 oral corticosteroid courses (OCS courses) and/or 2 ED visits during the 12 months (ED visits) before inclusion (Figure 1A). No association could be found with prior hospital admissions. As expected, allergic sensitizations to the most relevant allergens were associated with surrogate markers of atopy, such as atopic dermatitis (AD) and family history of atopy. In line with our current understanding that the skin is a major route for sensitization to egg, peanuts, and tree nuts, sensitization to these allergens, but not milk, was linked to AD. In contrast to other environmental allergens, the association with atopic dermatitis was present for Fel d 1, which may suggest that the skin is an important route of sensitization for this allergen. Altogether, these observations support the hypothesis that the skin is a key sensitizing organ both for food and for inhalant allergens that contribute to atopic disease progression.^{3,4}

To better understand sensitization profiles, component-resolved analysis was performed. Both ED visits and OCS courses were associated with sensitizations to seed storage proteins from peanut and

similar foods, including Ara h 1, Ara h 2, Ara h 3, Ara h 6, Gly m 6, Cor a 9, Cor a 11, and Cor a 14 in addition to the uteroglobin from cat and lipocalin from dog (Fel d 1 and Can f 1; Figure 1B). We also observed co-sensitization between allergens of related protein families from different sources, such as for peanut and soy (Figure S2). Both Ara h 1 and Fel d 1 were previously implicated as major risk allergens in the Swedish BAMSE and UK MAAS birth cohorts.⁵ Sensitizations identified to these allergens as early as 3–4 years old were useful for predicting development of respiratory allergy and asthma up to 16 years of age, in conjunction with environmental allergens from birch, grass, and dust mite.⁵ Sensitizations to the latter were less prominent in our cohort, which may reflect regional differences in environmental exposures.

To identify patterns of sensitization within our cohort, hierarchical clustering algorithms were applied, outlining three major subgroups: (a) a food and environmentally sensitized group, representative of the classical progression of the atopic march; (b) an environmentally sensitized group; and (c) a nonsensitized group (Figure 2). Recent reports present evidence that the mixed food and environmentally sensitized group has the greatest risk for persistent asthma development. Specifically, these reports are from the high-risk CAPS and MACS studies from Australia and the population-based LISAPLUS study from Germany.^{6,7} Differences between these clusters did not reach significance in clinical asthma severity and lung function parameters. However, re-assessment of TRACK scores after 3 months suggested that asthma control in the sensitized group (67%) more frequently improved compared to baseline than the nonsensitized group (33%), as reflected in their lack of improvement in TRACK scores at follow-up (Figure 1B). These data are consistent with reports that the nonsensitized group tends to be less responsive to standard corticosteroid asthma medications.⁸

In conclusion, we provide data describing a potentially high-risk preschool asthma cohort in Canada using a comprehensive approach capturing sensitization to allergens of paramount importance. By applying both allergen extracts and components, we identified that peanut and animal allergens from cat and dog were major allergen sources in this North American cohort of preschool asthmatics. In particular, seed storage proteins (Ara h 1, 2, 3, 6), uteroglobin from cat (Fel d 1), and lipocalin from dog (Can f 1) demonstrated the strongest linkage to clinical markers of asthma severity. This dataset provides detailed information on component-resolved diagnosis in preschool asthma and its potential contribution as biomarkers for stratification and endotyping in future prospective trials.

CONFLICTS OF INTEREST

Dr Eiwegger reports acting as site PI of a DBV-sponsored global study and grants from Innovation Fund Denmark, and is the Co-I or scientific lead in three investigator-initiated oral immunotherapy trials supported by the Allergy and Anaphylaxis Program at SickKids, outside of the submitted work. Dr Eiwegger serves as

associate editor for Allergy and is in the editorial board of the IAA and the review board of JACI. Dr Upton reports personal fees from Kaleo, personal fees from ALK, and acting as sub-investigator on clinical trials of DBV Therapeutics and Aimmune, outside the submitted work. Dr Maksym reports nonfinancial support from Thorasys Thoracic Medical Systems, Inc, outside the submitted work. The rest of the authors declare that they have no relevant conflicts of interest.

FUNDING INFORMATION

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KEYWORDS

allergen sensitization, preschool asthma, severe wheezing

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REFERENCES

1. Bisgaard H, Bonnelykke K. Long-term studies of the natural history of asthma in childhood. *J Allergy Clin Immunol*. 2010;126:187-197.

2. Dharma C, Lefebvre DL, Tran MM, et al. Patterns of allergic sensitization and atopic dermatitis from 1 to 3 years: effects on allergic diseases. *Clin Exp Allergy*. 2018;48:48-59.
3. Foong RX, Brough H. The role of environmental exposure to peanut in the development of clinical allergy to peanut. *Clin Exp Allergy*. 2017;47:1232-1238.
4. Du Toit G, Roberts G, Sayre PH, et al. Identifying infants at high risk of peanut allergy: the Learning Early About Peanut Allergy (LEAP) screening study. *J Allergy Clin Immunol*. 2013;131:135-143.
5. Wickman M, Lupinek C, Andersson N, 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.
6. Alduraywish SA, Standl M, Lodge CJ, et al. Is there a march from early food sensitization to later childhood allergic airway disease? Results from two prospective birth cohort studies. *Pediatr Allergy Immunol*. 2017;28:30-37.
7. Garden FL, Simpson JM, Marks GB, et al. Atopy phenotypes in the Childhood Asthma Prevention Study (CAPS) cohort and the relationship with allergic disease. *Clin Exp Allergy*. 2013;43:633-641.
8. Fitzpatrick AM, Bacharier LB, Guilbert TW, et al. Phenotypes of recurrent wheezing in preschool children: identification by latent class analysis and utility in prediction of future exacerbation. *J Allergy Clin Immunol Pract*. 2018;7:915-924.

SUPPORTING INFORMATION

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

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IL-6 and IL-1 β expression is increased in autologous serum skin test of patients with chronic spontaneous urticaria

To the Editors:

A number of studies have demonstrated elevated IL-6 serum level in patients with chronic spontaneous urticaria (CSU): higher in severe disease and in nonresponders to antihistamines, and remarkably decreased upon spontaneous remission.¹ Recent reviews have described IL-6 as a biomarker of CSU activity,² whereas IL-1 β has been poorly investigated, and data are contradictory.³⁻⁶ Two recent interesting studies have however shown, by microarray analyses, an upregulation of both IL-1 β expression and IL-6 expression in spontaneous urticarial wheals as compared to unaffected skin.^{7,8} In the present study, we confirm this upregulation, and moreover, we demonstrate, for the first time, that IL6 and IL1 β mRNA expression is significantly increased in positive autologous serum skin tests (ASST).

We compared IL6 and IL1 β mRNA expression in skin biopsies of negative ASST versus positive ASST, as well as in spontaneous urticarial wheals compared to uninvolved skin. We also analysed the expression of these cytokines in 48-hour PBMC cultures, in order to evaluate its

correlations with ASST response and autoimmune status (based on the history of autoimmune disease and/or presence of autoimmune antibodies). Seventy-two patients with CSU and 24 healthy controls (HCs) were included. Patient characteristics are summarized in Table S1.

We demonstrated that IL6 and IL1 β mRNA induction is significantly higher in positive as compared to negative ASST (7 negative ASST, 12 positive ASST, IL6 $P = 0.01$; IL1 β $P = 0.004$) (Figure 1A,B). We also observed a higher expression in spontaneous wheals (L) compared with noninvolved skin (NL) (IL6 $P < 0.0003$; IL1 β $P < 0.0003$, paired biopsies from 19 patients with CSU) (Figure 1A,B). These results corroborate published microarray reports.^{7,8} In addition, a positive correlation could be made between IL6 and IL1 β expression in skin biopsies (including positive/negative ASST and spontaneous wheals) of patients with CSU (38 paired biopsies, $P < 0.0001$) (Figure 1C).

IL6 and IL1 β mRNA expression in PBMCs of patients with CSU was also higher compared with HCs (67 patients with CSU and 24 HCs, IL6 $P = 0.02$; IL1 β $P = 0.0003$) (Figure 2A,B), and expression of

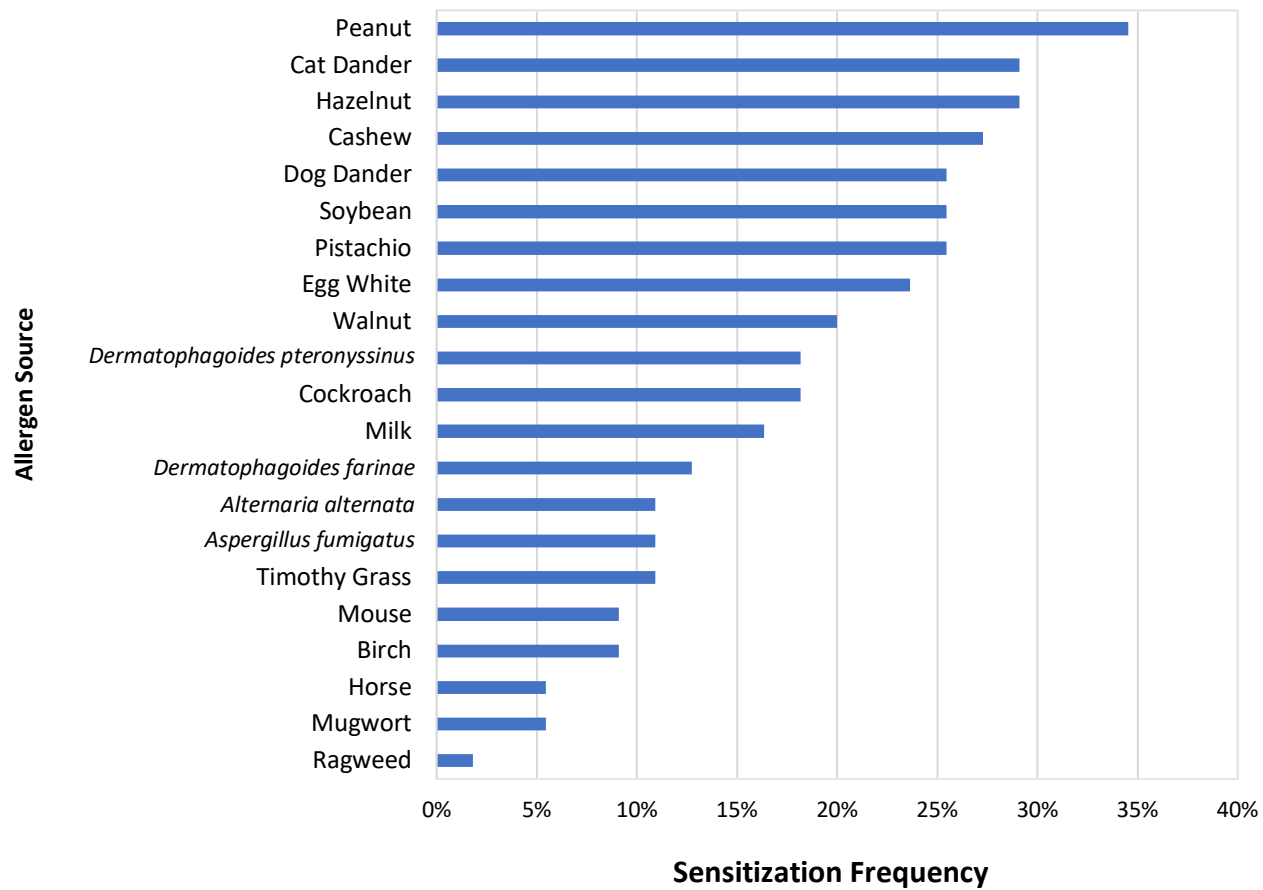


Figure S1

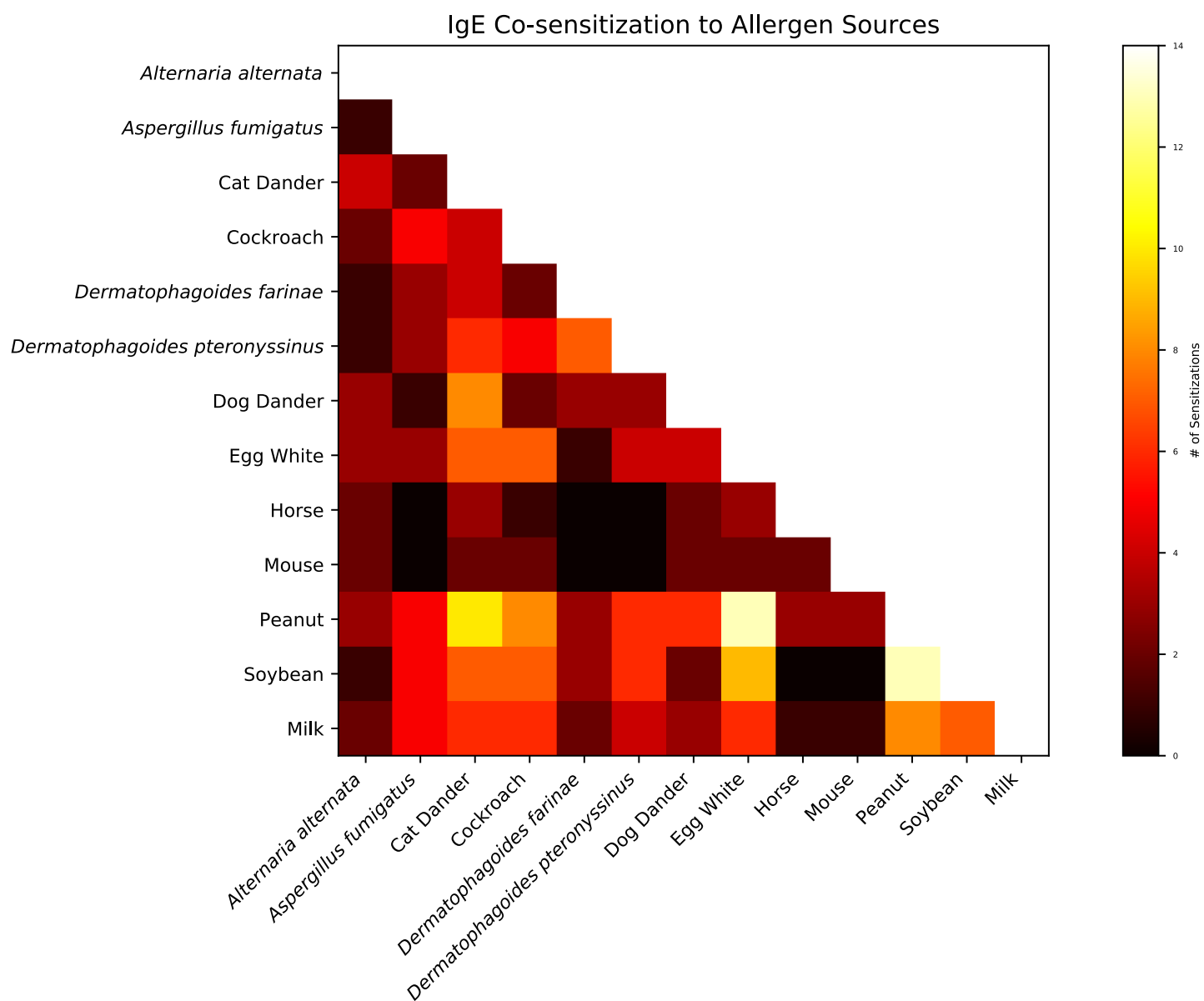


Figure S2

Supplementary Information – “Extract and component-specific sensitization patterns in Canadian moderate-to-severe preschool asthmatics”

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Keywords: **preschool asthma, allergen sensitization, severe wheezing**

Methods:

Study Design and Sample Collection

Eighty-seven preschool children (3-6 years) who presented with wheezing or asthma in the Emergency Department at the Hospital for Sick Children (Toronto, Canada) and had at least two episodes of wheezing in the past 12 months were prospectively enrolled in this observational study. Children were assessed at baseline, within 120 hours of discharge, and at the follow-up visit 3 months later. Clinical workup, symptom scoring, and lung function testing was performed at both visits. Allergic sensitization was tested at the follow-up visit. Skin prick tests (SPT) were performed to 14 allergenic sources. Peripheral blood samples were collected and serum isolated for specific IgE (sIgE) measurement with parent or legal guardian consent in a subset of 55 children from the study. This study is registered at ClinicalTrials.gov (NCT02743663) and the Hospital for Sick Children Research Ethics board approved the study (REB # 1000041089). Exclusion criteria for this study included premature birth or systemic co-morbidities.

Skin Prick Test

SPTs were performed and included 4 food allergens – peanut, cow's milk, soybean, egg white and 10 environmental allergens – *Alternaria alternata*, *Aspergillus fumigatus*, house dust mites (*Dermatophagoides pteronyssinus*, *Dermatophagoides farinae*), cat dander (cat hair and/or cat pelt), dog dander, mouse, horse, cockroach (ALK-Abelló). A positive SPT to an allergen source was defined as a mean wheal diameter of 2mm or greater.

Specific IgE Measurement

Specific IgE (sIgE) measurements were obtained with a chip-based nitrocellulose immunoassay composed of an array of 282 spotted allergens consisting of 156 extracts and 126 components (Allergy Explorer, Macroarray Dx, Vienna, Austria). Patient serum was incubated on the chip

with a diluent containing a CCD inhibitor for 2 hours, extensive washes in between steps, followed by incubation with an alkaline phosphatase-labelled anti-human IgE for 30 minutes and substrate for 8 minutes. After undergoing a colorimetric reaction and air drying, the intensity of the individual spots corresponding to the amount of bound IgE was captured by imaging with a benchtop camera reader. The sIgE was quantified based on an arbitrary calibration curve with dedicated software within a detectable range of 0.3-50 kUa/L (Raptor, Macroarray Dx). Major allergens within the chip were selected for analysis in this study as listed in Table S1. A positive sIgE measurement was defined as a calibrated value of 0.30 kUa/L or greater.

Analysis of Sensitization Profiles

A positive sensitization was defined as a positive SPT and/or sIgE measurement to an allergen source or related allergen component. Heatmaps were then generated to identify correlations between sensitizations with clinical features and risk factors of asthma. Hierarchical clustering was also performed on SPT and sIgE sensitization data. Analysis and graphs were generated with Python and Microsoft Excel.

Clinical Assessment of Asthma and Allergic Diseases

Clinical assessments were performed by a trained pediatric respirologist. The diagnosis of asthma was made according to the Canadian Thoracic Society criteria: >2 discrete episodes of wheezing within the past year responsive to a bronchodilator, and no other relevant diagnoses.

Medical history was reviewed to obtain information regarding wheezing and asthma exacerbations at baseline and follow-up in terms of the number of oral corticosteroid courses taken, number of emergency department visits, hospitalizations. Atopic history, including atopic dermatitis, food allergy and parental allergic history was obtained by parental report of symptoms via questionnaire.

The Test for Respiratory and Asthma Control in Kids (TRACK) score was generated by completion of a validated questionnaire for assessing asthma symptoms and control in preschool children. The TRACK score ranges between 0-100, and a TRACK score less than 80 suggests poorly controlled asthma while a TRACK score over 80 indicates controlled asthma.

Supplementary Tables

Table S1: SPT and allergen array extracts and components.

Allergenic Source	SPT Extract	Array Extract	Array Component
Perennial Allergens			
<i>Alternaria alternata</i> (<i>Alt a</i>)	<i>Alternaria tenuis</i> (ALK #1058942)	<i>Alt a</i>	<i>Alt a 1</i>
<i>Aspergillus fumigatus</i> (<i>Asp f</i>)	<i>Asp f</i> (ALK #1060330)	<i>Asp f</i>	<i>Asp f 3, Asp f 4, Asp f 6</i>
Cockroach / <i>Blattella germanica</i> (<i>Bla g</i>) & <i>Periplaneta americana</i> (<i>Per a</i>)	Cockroach (ALK #1041378)	<i>Bla g</i> <i>Per a</i>	<i>Bla g 1, Bla g 2, Bla g 4, Bla g 5</i> <i>Per a 7</i>
<i>Dermatophagoides pteronyssinus</i> (<i>Der p</i>)	<i>Dermatophagoides pteronyssinus</i> (ALK #1040984)	<i>Der p</i>	<i>Der p 1, Der p 2, Der p 5, Der p 7, Der p 10, Der p 11, Der p 23</i>
<i>Dermatophagoides farinae</i> (<i>Der f</i>)	<i>Dermatophagoides farinae</i> (ALK #1040935)	<i>Der f</i>	<i>Der f 1, Der f 2</i>
Cat / <i>Felis domesticus</i> (<i>Fel d</i>)	Cat Hair (ALK # 1042363) Cat Pelt (ALK #1042028)	<i>Fel d</i>	<i>Fel d 1, Fel d 2, Fel d 4</i>
<i>Dog</i> / <i>Canis familiaris</i> (<i>Can f</i>)	Dog Epithelium (ALK #1040955)	<i>Can f</i>	<i>Can f 1, Can f 2, Can f 3</i>
Mouse / <i>Mus musculus</i> (<i>Mus m</i>)	Mouse		<i>Mus m 1</i>
Horse / <i>Equus caballus</i> (<i>Equ c</i>)	Horse	<i>Equ c</i> _epithelia	<i>Equ c 1</i>
Environmental Allergens			
Ragweed / <i>Ambrosia artemisiifolia</i> (<i>Amb a</i>)	Ragweed Mix (ALK #1041474)	<i>Amb a</i>	<i>Amb a 1, Amb a 4</i>
Mugwort / <i>Artemisia vulgaris</i> (<i>Art v</i>)		<i>Art v</i>	<i>Art v 1, Art v 3</i>
Birch / <i>Betula verrucosa</i> (<i>Bet v</i>)		<i>Bet v</i>	<i>Bet v 1, Bet v 2, Bet v 6</i>
Timothy Grass / <i>Phleum pratense</i> (<i>Phl p</i>)		<i>Phl p</i>	<i>Phl p 1, Phl p 2, Phl p 5.0101, Phl p 6, Phl p 7, Phl p 12</i>
Food Allergens			
Peanut / <i>Arachis hypogaea</i> (<i>Ara h</i>)	Peanut (ALK #1041678)	<i>Ara h</i>	<i>Ara h 1, Ara h 2, Ara h 3, Ara h 6, Ara h 8, Ara h 9</i>
Cow Milk / <i>Bos domesticus</i> (<i>Bos d</i>)	Whole Cow's Milk (ALK #1040876)	<i>Bos d</i>	<i>Bos d 4, Bos d 5, Bos d 6, Bos d 8</i>
Chicken Egg White / <i>Gallus domesticus</i> (<i>Gal d</i>)	Egg White (ALK #1041013)	<i>Egg_white</i>	<i>Gal d 1, Gal d 2, Gal d 3, Gal d 4</i>
Soybean / <i>Glycine max</i> (<i>Gly m</i>)	Soybean (ALK #1041956)	<i>Gly m</i>	<i>Gly m 4, Gly m 5, Gly m 6, Gly m 8</i>
Walnut / <i>Juglans regia</i> (<i>Jug r</i>)		<i>Jug r_nut</i> <i>Jug r_pollen</i>	<i>Jug r 1, Jug r 2</i>
Pistachio / <i>Pistacia vera</i> (<i>Pis v</i>)		<i>Pis v</i>	
Hazelnut / <i>Corylus avellana</i> (<i>Cor a</i>)		<i>Cor a_hazel</i> <i>Cor a_pollen</i>	[‡] <i>Cor a 1.0103, [†]Cor a 1.0401, Cor a 8, Cor a 9, Cor a 11, Cor a 14</i>
Cashew / <i>Anacardium occidentale</i> (<i>Ana o</i>)		<i>Ana o</i>	<i>Ana o 3</i>

Table S2: Demographics and clinical characteristics of participants.

	Component & SPT Data		Complete Cohort	
	Summary	Number of Subjects	Summary	Number of Subjects
<u>Demographics</u>				
Age at inclusion (Mean [SD])	4.6 (0.9)	55	4.4 (0.9)	87
Sex, male (%)	66%	36/55	62%	54/87
<u>Clinical Characteristics of Asthma Severity</u>	Mean (SD)	N	Mean (SD)	N
Hospitalizations in Past Year at Inclusion	0.4 (0.7)	55	0.4 (0.7)	86
ED Visits in Past Year at Inclusion	4.7 (3.55)	55	4.1 (3.39)	87
Oral Corticosteroid Courses in Past Year at Inclusion	1.92 (1.33)	55	1.6 (2.81)	87
TRACK Score at Inclusion	50.9 (24.1)	54	51.4 (22.3)	86
TRACK Score at Follow Up Visit	62.0 (21.7)	55	65.7 (20.5)	82
<u>Atopic History</u>	%	n/N	%	n/N
Atopic Dermatitis	71%	39/55	74%	63/85
Food Allergy	27%	15/55	28%	23/83
Parental History of Allergy/Atopy	73%	40/55	77%	67/87
<u>Sensitizations</u>	Component/ SPT %	n/N	SPT %	n/N
Sensitized (Any allergen source)	67%	37/55	57%	43/76
Environmental Allergens	62%	34/55	47%	36/76
Food Allergens	44%	24/55	26%	20/76
Monosensitized (1 allergen source)	7%	4/55	24%	18/76
Oligosensitized (2-4 allergen sources)	24%	13/55	26%	20/76
Oligosensitized – Environmental only	15%	8/55	12%	9/76
Oligosensitized – Food only	0%	0/55	4%	3/76
Oligosensitized – Environmental & Food	9%	5/55	11%	8/76
Polysensitized (> 4 allergen sources)	36%	20/55	7%	5/76
Polysensitized – Environmental only	4%	2/55	0%	0/76
Polysensitized – Food only	4%	2/55	0%	0/76
Polysensitized – Environmental & Food	29%	16/55	7%	5/76
<u>Asthma Medications</u>	%	n/N	%	n/N
OCS Usage – At Inclusion	93%	51/55	93%	81/87
OCS Usage – Follow Up	36%	20/55	29%	24/82
ICS Usage – At Inclusion	89%	49/55	93%	81/87
ICS Usage – Follow Up	87%	48/55	89%	73/82
Among ICS Users - Continuously on ICS at Inclusion	39%	19/49	34%	30/87
Among ICS Users - Continuously on ICS at Follow Up	58%	28/48	55%	40/73

Abbreviations: ED, emergency department; OCS, oral corticosteroid; ICS, inhaled corticosteroid; SPT, Skin Prick Test, TRACK, Test for Respiratory and Asthma Control in Kids. Data presented as n/N where indicated, where N is the total number of patients with available observations for that variable.

Table S3: Clinical characteristics of sensitized vs. non-sensitized patients.

	Sensitized (N = 37)		Non-Sensitized (N = 18)	
<u>Demographics</u>	Summary	Number of Subjects	Summary	Number of Subjects
Age at inclusion (Mean [SD])	4.4 (0.85)	37	4.2 (0.95)	18
Sex, male (%)	65%	24/37	67%	12/18
<u>Clinical Characteristics of Asthma Severity</u>	Mean (SD)	N	Mean (SD)	N
Hospitalizations in Past Year at Inclusion	0.3 (0.5)	36	0.4 (0.9)	18
ED Visits in Past Year at Inclusion	4.2 (3.2)	37	5.3 (4.2)	18
Oral Corticosteroid Courses in Past Year at Inclusion	1.8 (1.0)	37	2.1 (1.8)	18
TRACK Score at Inclusion	50.3 (23.3)	36	52.2 (26.2)	18
TRACK Score at Follow Up Visit	64.5 (20.8)	37	56.9 (23.3)	18
<u>Atopic History</u>	%	n/N	%	n/N
Atopic Dermatitis	78%	29/37	56%	10/18
Food Allergy	32%	12/37	17%	3/18
Parental History of Allergy/Atopy	73%	27/37	72%	13/18
<u>Asthma Medications</u>	%	n/N	%	n/N
OCS Usage – At Inclusion	95%	35/37	89%	16/18
OCS Usage – Follow Up	32%	12/37	44%	8/18
ICS Usage – At Inclusion	89%	33/37	100%	18/18
ICS Usage – Follow Up	81%	30/37	100%	18/18
Among ICS Users - Continuously on ICS at Inclusion	32%	12/37	44%	8/18
Among ICS Users - Continuously on ICS at Follow Up	46%	17/37	61%	11/18

Abbreviations: ED, emergency department; OCS, oral corticosteroid; ICS, inhaled corticosteroid; TRACK, Test for Respiratory and Asthma Control in Kids. Data presented as n/N where indicated, where N is the total number of patients with available observations for that variable.

Table S4: Sensitization reported by skin prick test vs. allergen array.

Sensitizations	Skin Prick Test %	n/N	Allergen Array %	n/N
Sensitized (Any allergen source)	54%	28/52	67%	37/55
Environmental Allergens	46%	24/52	62%	34/55
Food Allergens	25%	13/52	44%	24/55
Monosensitized (1 allergen source)	19%	10/52	11%	6/55
Oligosensitized (2-4 allergen sources)	27%	14/52	25%	14/55
Oligosensitized – Environmental Only	13%	7/52	15%	8/55
Oligosensitized – Food Only	4%	2/52	2%	1/55
Oligosensitized – Environmental + Food Sources	10%	5/52	9%	5/55
Polysensitized (> 4 allergen sources)	8%	4/52	31%	17/55
Polysensitized – Environmental Only	0%	0/52	0%	0/55
Polysensitized – Food Only	0%	0/52	2%	1/55
Polysensitized – Environmental + Food Sources	8%	4/52	29%	16/55

Supplementary Figure Legends

Fig S1: Sensitization frequency to allergen sources observed within severe wheezing preschoolers (n = 55). Positive sensitization was defined as a SPT $\geq$ 2mm and/or array sIgE $\geq$ 0.3 kUa/L to at least one extract or component mapped to a given allergen.

Fig S2: IgE co-sensitizations to allergen extracts. Scale indicates number of cases with concurrent positive sensitizations.