Development of Sensitization to Multiple Allergen Molecules from Preschool to School Age Is Related to Asthma

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Keywords

Allergen molecules · Allergic sensitization · Asthma · Longitudinal · Polysensitization · Preschool wheeze

Abstract

Introduction: Allergic sensitization in early life has been identified as a strong risk factor for subsequent asthma in childhood. It is still unclear why only a part of sensitized children develop asthma, and the role of specific allergen molecules in asthma pathogenesis is ambiguous [*Pharmacol Ther.* 2009 Feb;121(2):174–84]. We assessed the sensitization to multiple allergen molecules longitudinally and explored its relation to persistent asthma at 7 years. **Methods:** Seventy-two children included during an acute wheezing episode (cases) were followed prospectively from early preschool age (EPA) to age 7, and compared to 43 healthy controls at EPA. Allergen molecules were analyzed at EPA and age 7 using ImmunoCAP Solid-phase Allergen Chip (ISAC). Asthma diagnosis at 7 years was based on symptoms, med-

ication, and spirometry. **Results:** At EPA, cases compared to controls showed a tendency toward having a higher prevalence of allergic sensitization (23.6% vs. 9.3%, $p = 0.055$). The prevalence of sensitization increased in cases from EPA to 7 years (23.6% vs. 38.9%; $p = 0.048$) as well as the median number (range) of immunoglobulin E (IgE)-reactive molecules 3 (3–14) versus 6.5 (1–21); $p = 0.024$. Sensitization to each additional molecule from EPA to the age of 7 was significantly related to asthma at 7 (OR = 1.25, 95% confidence interval [1.01, 1.54]). **Conclusion:** Polysensitization, assessed by allergen molecules, had a significant impact on persistent asthma at school age. The extent of sensitization, illustrated by molecular spreading from preschool to school age, was related to asthma diagnosis at 7 years in children with a history of wheezing at early life.

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Published by S. Karger AG, Basel

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Edited by: H.-U. Simon, Bern.

Introduction

Asthma is a highly heterogeneous disease, characterized by variable airflow obstruction, bronchial hyperresponsiveness, airway inflammation and in some cases airway remodeling [1]. The etiological mechanisms involved in the distinct phenotypical expressions of asthma constitute an issue of ambiguity.

Asthma is the most common chronic disease of childhood and is often preceded by respiratory illness with wheeze in preschool age [2]. Two-thirds of preschool wheezers outgrow their symptoms by the age of 6 years and the remaining third develop asthma [3–5]. Although the role of environmental and host-specific agents in asthma pathogenesis has been studied extensively, the factors that determine the persistence of asthma in later childhood remain unclear [2, 6, 7]. A number of epidemiological studies have shown a strong relationship between allergic sensitization and asthma [8–11]. Nevertheless, this relationship is inconsistent, and allergic sensitization seems to be neither necessary nor sufficient for the development of asthma [9, 12–15].

Allergic sensitization can be measured by skin prick tests or allergen-specific immunoglobulin E antibodies (IgE) in serum. IgE to either extract from whole allergen sources or to individual allergen molecules can be measured. The latter helps differentiate genuine sensitization against a certain allergen source from cross-reactivity due to homology between proteins from different allergen sources [16–18]. There are several techniques for molecular allergy diagnostics. ImmunoCAP Solid-phase Allergen Chip (ISAC) is a comprehensive platform composed of 112 micro-arrayed allergens and utilizes only minute amounts of serum [17, 19].

Sensitization to airborne or food allergens in early life has been identified as strong risk factors for subsequent asthma diagnosis in a number of cohort studies [20–24]. However, it remains unclear why only a part of sensitized

children develop asthma and which allergen molecules are involved in the asthma pathogenesis at an early stage. There can be numerous sensitizing profiles and distinct combinations of allergen molecules that differ in their association with asthma [25–27]. Therefore, there is a need to further explore allergic sensitization on a molecular allergen level and clarify its impact on the onset and persistence of asthma.

The “Gene Expression in Wheezing and Asthmatic Children” (GEWAC) study started in 2008 and aimed to shed light on the complex etiology of preschool wheeze. Significant associations between subnormal levels of vitamin D [28], elevated chitinase-like protein (YKL-40) levels [29] as well as early infection with specific serotypes of RV [30] and acute wheeze have previously been shown in GEWAC cohort. In the current study, we assessed the sensitization profiles to a comprehensive panel of allergen molecules from early preschool age (EPA) to school age and studied their relation to persistent asthma at 7 years of age in the GEWAC cohort.

Materials and Methods

Study Design and Cohort

GEWAC is a longitudinal case-control study consisting of 156 cases and 102 age-matched healthy controls, as previously described [28, 29]. The cases (age 6–48 months) were enrolled at the pediatric emergency ward at Astrid Lindgren’s Children’s Hospital, Stockholm, Sweden, between 2008 and 2012 when presenting with acute wheeze. They came to a first revisit approximately 3 months later, and have been followed prospectively until 7 years of age. The age-matched controls were recruited from the surgical day-care ward during the same time period before going through minor surgery. The controls were assessed at inclusion, and at the age of 7 years. Inclusion and exclusion criteria are found in Table 1.

The study population of the current study comprises 72 cases and 43 controls. Sera for analyses of allergen molecules were collected both at the Early Preschool Age (EPA, sera available from either the time of inclusion [$N = 7$] or the first revisit [$N = 65$]) and at 7 years, when the children were also assessed regarding asthma

Table 1. Inclusion and exclusion criteria, GEWAC study

	Inclusion criteria	Exclusion criteria
Cases ($N = 156$)	Age of 6–48 months Acute symptoms of wheeze	Prematurity (birth before 36 gestational weeks) Chronic disease Simultaneous complications (e.g., sepsis, bacterial pneumonia)
Controls ($N = 102$)	Age of 6–48 months	Prematurity (birth before 36 gestational weeks) History of bronchial obstruction and asthma or known sensitization to airborne allergens

Fig. 1. Flowchart GEWAC population included in the current study; the number of the cases and controls included in the different timepoints. #The group of cases with available sera at the first revisit and at 7 years ($n = 65$) was merged with 7 cases that provided sera at the emergency visit and at 7 years. Accordingly, the first timepoint of the longitudinal assessment of the cases is referred as the EPA. *Nasopharyngeal samples, **Complete Blood Counts.

diagnosis. Sera from the controls were available at the time of inclusion ($N = 43/43$) and at 7 years ($N = 27/43$) (Fig. 1).

Sample Collection

A standardized questionnaire regarding ethnicity, lifestyle, family history of asthma and allergies, breastfeeding, exposure to tobacco smoke, pets and previous infections, occurrence of atopic and asthmatic symptoms, use of asthma medication was filled out at the first revisit of cases and at inclusion of controls. Another

standardized questionnaire regarding atopic symptoms as well as occurrence, frequency and duration of asthmatic symptoms and use of asthma medication during the preceding 12 months along with The Asthma Control Test (ACT) was filled out at the 7 years' follow-up.

Blood samples were collected from the cases and controls at each visit and analyzed at the laboratories of Clinical Chemistry, and of Clinical Immunology, Karolinska University Hospital, Stockholm Sweden. Serum analysis for 112 allergen molecules us-

Table 2. An overview of the main characteristics of the controls as well as the cases at EPA and the age of 7 years

Variables at baseline (EPA)	Controls (N = 43)	Cases (N = 72) at EPA	p value cases and controls at EPA	Cases without asthma at 7 years (N = 23)	Cases with asthma at 7 years (N = 49)	p value cases with and without asthma at age 7
Age (mean value in months)	27	23	0.027	20.5	24	0.14
Gender (male), % (N)	79.1 (34)	66.7 (48)	0.155	73.9 (17)	63.3 (31)	0.372
Heredity asthma, ^a % (N)	10.0 (4)	40.0 (28)	0.001	31.8 (7)	43.8 (21)	0.344
Heredity atopy, ^a % (N)	55.0 (22)	72.9 (51)	0.057	68.2 (15)	75.0 (36)	0.552
Caucasian mother or father, ^a % (N)	73.7 (28)	87.3 (62)	0.074	95.5 (21)	83.7 (41)	0.257
Exclusive breastfeeding at 4 months, ^a % (N)	78.0 (32)	53.6 (37)	0.010	72.7 (16)	44.7 (21)	0.029
Smoking during pregnancy, ^a % (N)	0.0 (0)	9.9 (7)	0.046	4.5 (1)	12.2 (6)	0.423
Previous verified RSV infection, ^a % (N)	2.4 (1)	25.4 (18)	0.002	27.3 (6)	24.5 (12)	0.803
Allergic sensitization at EPA/inclusion, % (N ^b)	9.3 (4)	23.6 (17)	0.055	17.4 (4)	26.5 (13)	0.395
Allergic sensitization in controls/cases aged <24 months at inclusion/EPA, % (N)	11.1 (2/18)	15.2 (7/46)	1.000	6.3 (1/16)	20.0 (6/30)	0.394
Allergic sensitization in controls/cases aged ≥24 months at inclusion/EPA, % (N)	8.0 (2/25)	38.5 (10/26)	0.010	42.9 (3/7)	36.8 (7/19)	1.000
Allergen molecules, n per sensitized individual ^b ; median value (range)	1.5 (1–2)	3 (1–14)	0.082	1.5 (1–3)	3 (1–14)	0.079
Sensitization to ≥2 molecules, % (N)	4.6 (2)	18.1 (13)	0.039	8.7 (2)	22.4 (11)	0.202
Total IgE (median values, kU/L)	18.3	24.51	0.789	12.23	34.7	0.341
Airborne allergy symptoms, ^a % (N)	0.0 (0)	5.6 (4)	0.295	0 (0)	8.2 (4)	0.303
Food allergy symptoms, ^a % (N)	4.9 (2)	11.3 (8)	0.321	4.5 (1)	14.3 (7)	0.420
Eczema, ^a % (N)	7.3 (3)	21.1 (15)	0.055	18.2 (4)	22.4 (11)	0.763

Variables at the age of 7 years	Controls at the age of 7 years (N = 27)	Cases at the age of 7 years (N = 72)	p value cases and controls at 7 years	Cases without asthma at 7 years (N = 23)	Cases with asthma at 7 years (N = 49)	p value; cases with and without asthma at age 7
Allergic sensitization at age 7, % (N ^b)	22.2 (6)	38.9 (28)	0.120	26.1 (6)	22 (44.9%)	0.127
Allergen molecules, n per sensitized individual ^b ; median value (range) at age 7	1.5 (1–7)	6.5 (1–21)	0.017	4 (1–7)	10.5 (1–21)	0.059
Sensitization to ≥2 molecules at age 7, % (N)	11.1 (3)	33.3 (24)	0.027	17.4 (4)	40.8 (20)	0.049
Airborne allergy symptoms at age 7, % (N)	0 (0)	18.1 (13)	0.017	0.0 (0)	26.5 (13)	0.006
Food allergy symptoms at age 7, % (N)	3.7 (1)	11.1 (8)	0.437	0.0 (0)	16.3 (8)	0.049
Eczema at age 7, % (N)	3.7 (1)	9.7 (7)	0.441	0 (0)	14.3 (7)	0.089
Asthma at the age of 7 years, % (N)	3.7 (1)	68.1 (49)	<0.001	100 (23)	100 (49)	–

^a p values for comparisons between cases and controls as well as between cases with and without asthma at the age of 7 years. RSV, respiratory syncytial virus. ^a 69–71 cases/38–41 controls.

^b Cases and controls having an allergen-specific IgE level ≥0.3 ISU against at least one allergen molecule.

ing ImmunoCAP ISAC (Thermo Fisher Scientific, Uppsala, Sweden) was performed at the Thermo Fisher Scientific laboratory. The allergen-specific IgE level is expressed semiquantitatively in ISU (ISAC Standardized Units). The cutoff IgE ≥ 0.3 ISU was considered positive.

Spirometry and airway reversibility were performed using the Medikro Pro spirometer at the 7 years' follow-up. Reversibility was evaluated 15 min after inhalation with 400 μ g salbutamol using a spacer. The patient's technique and quality of the spirometry were evaluated according to international guidelines [31]. If the test criteria were not fulfilled, the test result was not included in the analyses. The legal guardians were advised not to give their children asthma medication (salbutamol, corticosteroids or leukotriene antagonists) up to 24 h before the revisit at 7 years of age.

Exposures and Outcomes

Heredity; history of asthma, eczema, inhalant, or food allergy in any of the parents or siblings. Heredity for atopy denotes history of at least one type of allergy or eczema in any of the parents or siblings.

Breastfeeding; exclusive breastfeeding during at least 4 months in infancy.

Tobacco smoke in utero; if the mother has smoked during pregnancy regardless of trimester.

Allergy symptoms; airborne allergy symptoms including rhinitis (sneezing, runny or blocked nose) and/or conjunctivitis, food allergy, and eczema (having dry skin in combination with a pruritic rash for at least 2 weeks) during the last 12 months as reported in the questionnaire at the respective revisit.

Allergic sensitization was defined as having an allergen-specific IgE level ≥ 0.3 ISU against at least one allergen molecule.

Asthma Definition

The definition of asthma at 7 years of age was based on Global Initiative for Asthma (GINA) guidelines [32] and included one mandatory criterion (a diagnosis of asthma by the study doctor, a pediatric allergist [KSH]) and 3 additional criteria: lower respiratory symptoms, medication for treatment of wheeze the preceding 12 months or airway reversibility $>12\%$ after the use of bronchodilator with salbutamol. Lower respiratory symptoms were defined as cough, shortness of breath, and nocturnal awakening caused by wheezing for 5 days or longer the preceding 12 months. All children that fulfilled the compulsory and at least one of the optional criteria (symptoms, medication or reversibility) were classified as having asthma.

Statistical Analyses

SPSS version 26 (IBM) was used. The prevalence of studied variables, expressed in percent of the total number of the available observations and the median number of positive IgE responses with ranges, were calculated for controls and cases at different timepoints. The median values of allergen molecule-specific IgE were calculated in groups of individuals sensitized to the respective molecule. 112 allergen molecules were ranked by prevalence and median value in the cases at 2 different time points; at EPA, and at 7 years. Combined ranking was then applied by ranking the sum of ranks for prevalence and median value for each allergen molecule. The χ^2 test was used to analyse for significant proportional differences in categorical characteristics between 2 groups, and Fisher's exact test was used on small sample sizes. An unpaired *t*

test was used for numerical variables and a nonparametric test, Mann-Whitney, was used when assumptions for the unpaired *t* test were not met. *p* value <0.05 was considered significant. Odds ratios were calculated by univariate logistic regression and 95% confidence intervals (95% CI). Probability curves for asthma at 7 years of age based on the number of sensitizing allergen molecules (whichever of the 112 molecules) were derived from the results of a univariate logistic regression.

Results

Baseline Characteristics and Assessment of the Allergic Sensitization in Cases Compared to Controls at EPA

The study population consists of 72 cases who were assessed at EPA and age 7 as well as 43 controls investigated at EPA. No significant differences in demography, studied exposures, and atopic symptoms were observed between the 72 cases included and the 84 cases excluded from the current study population (online suppl. Table S1; see www.karger.com/doi/10.1159/000521324 for all online suppl. material). The included controls (*N* = 43) were significantly older (27 vs. 17 months, *p* < 0.001) and had a lower prevalence of heredity for asthma (10% vs. 27%, *p* = 0.037) than the excluded controls (*N* = 59) (online suppl. Table S1). Cases (*N* = 72) and controls (*N* = 43) differed significantly with respect to heredity for asthma, exposure to tobacco smoke during pregnancy, exclusive breastfeeding at 4 months, and previous Respiratory Syncytial Virus infections (Table 2). The controls had no knowledge of sensitization to airborne molecules which was a prerequisite for inclusion (Table 1).

At EPA, cases compared to controls showed a tendency toward having a higher prevalence of allergic sensitization (23.6% vs. 9.3%, *p* = 0.055) and being sensitized to a higher median number of molecules (3 [1–14] vs. 1.5 [1–2], *p* = 0.082, Table 2). Furthermore, sensitization to ≥ 2 molecules was noticed in a higher proportion of cases compared to controls (18.1% vs. 4.6%, *p* = 0.039). In total, sensitization against 37 different molecules was observed among cases, whereas the corresponding number in the controls was 6.

Longitudinal Assessment of Allergic Sensitization Profiles in the Preschool Wheezers (Cases)

Demography and data on exposure and serum analyses at EPA and 7 years in the 72 cases are demonstrated in Table 2. The prevalence of allergic sensitization increased from 23.6% at EPA to 38.9% at 7 years (*p* = 0.048, Table 2, shown in Fig. 2a). The median number of molecules with IgE reactivity in each sensitized case increased

Fig. 2. Trajectory of allergic sensitization in cases from EPA to the age of 7. The prevalence of allergic sensitization, sensitization to airborne molecules*, food molecules** and to each of the highest ranked molecules at EPA and at 7 years in all included cases ($N = 72$) (a), in cases without asthma diagnosis at 7 years ($N = 23$) (b), and in cases with asthma diagnosis at 7 years ($N = 49$) (c). *Sensitized to at least one of the airborne molecules; Phl p 1, Phl p 2, Phl p 4, Phl p 5, Phl p 6, Phl p 11, Phl p 12, Aln g 1, Bet v 1, Cor a 1.01, Cry j 1sens, Cup a 1, Ole e 7, Ole e 9, Pla a 1, Pla a 2, Pla a 3, Art v 1, Art v 3, Mer a 1, Can f 1, Can f 2, Can f 3, Can f 5, Equ c 1, Fel d 1, Fel d 2, Fel d 4sens, Mus m 1, Alt a 6, Asp f 3, Asp f 6, Der f 1, Der f 2, Der p 1, Der p 2, Der p 10, Bla g 2, and Bla g 7. **Sensitized to at least one of the food molecules; Gal d 1, Gal d 2, Gal d 3, Gal d 5, Bos d 4, Bos d 5, Bos d 6, Bos d 8, Bos d L, Pen m 1, Pen m 2, Ana o 2, Ber e 1, Cor a 1.04, Cor a 8, Cor a 9, Jug r 1, Jug r 3, Jug r 2, Jug r 3, Ses i 1, Ara h 1, Ara h 2, Ara h 3, Ara h 6, Ara h 8, Ara h 9, Gly m 4, Gly m 5, Gly m 6, Act d 1, Act d 2, Act d 8, Api g 1, Mal d 1, Pru p 1, and Pru p 3.

from 3 (1–14) at EPA to 6.5 (1–21) at age 7 ($p = 0.024$, Fig. 3a). The prevalence of sensitization to ≥ 2 molecules increased from 18.1% to 33% ($p = 0.036$) and the total number of allergen-specific IgE responses increased from 37 at EPA to 65 at age 7. Fifty-four percent ($N = 39$) of the cases were sensitized to the same number of molecules at EPA and age 7, whereas 39% ($N = 28$) were sensitized to more molecules and 7% (5) to fewer molecules at 7 years.

When applying combined ranking, the highest ranked molecule was Mal d 1 (apple) at EPA, and Bet v 1 (birch) at 7 years (online suppl. Tables S2, S3). Ara h 2 (peanut) and Bet v 1 were among the 3 highest ranked molecules at both timepoints (online suppl. Tables S2, S3). The prevalence of IgE reactivity to the most prevalent air-

borne molecules increased significantly from EPA to 7 years' age (Bet v 1; 4.2% vs. 20.8%, $p = 0.002$, Fel d 1 [cat]; 2.8% vs. 13.9%, $p = 0.016$, Phl p 1 [timothy grass]; 0% vs. 19.4%, $p < 0.001$) whereas sensitization to peanut molecules (Ara h 1, Ara h 2, Ara h 3 and Ara h 6), developed at EPA, did not change significantly over time ($p = 1.000$, $p = 1.000$, $p = 0.719$, $p = 1.000$ respectively, Fig. 2a).

Assessment of the Allergic Sensitization in the Preschool Wheezers at EPA in Relation to Asthma at 7 Years (Cases)

The prevalence of asthma at the age of 7 years was 68.1% among the cases. Although no significant difference in the prevalence of allergic sensitization at EPA

Fig. 3. Box plots for the number of molecules with IgE reactivity per individual at EPA and at 7 years among all of the sensitized cases (a), and the sensitized cases without and with asthma at 7 years (b).

was noticed between the groups of cases with and without asthma at 7 years (Table 2), sensitization to each additional molecule from EPA to the age of 7 was significantly related to asthma at 7 years (OR 1.25, 95% CI [1.01, 1.54], $p = 0.04$; Fig. 4a). Interestingly, the median number of molecules with IgE reactivity increased significantly from 3 (1–14) at EPA to 10.5 (1–21) at age 7, in each sensitized case with asthma at 7 years ($p = 0.038$, Table 2; Fig. 3b) whereas no significant change was noticed in cases without asthma at 7 years ($p = 0.257$, Table 2; Fig. 3b). Furthermore, a significantly greater increase in the number of IgE reactivities to allergen molecules from EPA to 7 years was noticed in cases sensitized against Ara h 2 ($p = 0.003$), Ara h 6 ($p = 0.003$), Bet v 1 ($p = 0.042$), or Fel d 1 ($p = 0.033$) at EPA compared to those not being sensitized against any of these molecules, which were among the highest ranked molecules at both time points (online suppl. Table S2). In addition, all children sensitized to ≥ 1 of the peanut molecules Ara h 1, Ara h 2, Ara h 3, and Ara h 6 at EPA ($N = 7$, 9.7%) received an asthma diagnosis at the age of 7 ($p = 0.089$, Fig. 2b, c).

Assessment of the Allergic Sensitization in the Preschool Wheezers at 7 Years and Its Relation to Asthma Diagnosis at 7 Years (Cases)

The prevalence of allergic sensitization at 7 years did not differ significantly between the asthma and the non-asthma group at 7 years ($p = 0.127$, Table 2). However, the prevalence of sensitization to Bet v 1 (20.8%) at 7 years was significantly higher among the cases with asthma than those without asthma ($N = 14$ [28.6%] vs. $N = 1$ (4.3%), $p = 0.027$, online suppl. Table S3; Fig. 2b, c). Moreover, the number of sensitizing molecules in each case at 7 years (6.5 [1–21]) was significantly associated to asthma at the same age (OR 1.20, 95% CI [1.02, 1.42], $p = 0.043$; Fig. 4b). Notably, the cases with sensitization to ≥ 6 molecules at 7 years ($N = 15$, 20.8%) were more likely to have asthma ($p = 0.027$, OR = 8.80, 95% CI [1.08, 71.70]) than those sensitized to < 6 molecules.

Discussion

In the cross-sectional part of our study, the prevalence of sensitization to ≥ 2 molecules at EPA was significantly higher in children with preschool wheezing compared to the healthy controls. The longitudinal approach focused

Fig. 4. a The difference in the number of allergen molecules with IgE reactivity per individual between EPA and the age of 7 and probability for asthma at 7 years. OR 1.248, 95% CI (1.011–1.540), $p = 0.039$; calculated by logistic regression in cases ($N = 72$). **b** The number of allergen molecules with IgE reactivity per individual at 7 years and probability for asthma at 7 years. OR 1.195, 95% CI (1.006–1.419), $p = 0.043$; calculated by logistic regression in cases ($N = 72$).

on the assessment of the sensitization profiles in children with a history of wheezing at preschool age, their development over time and subsequent risk for asthma at school age. Using the comprehensive ISAC platform, we noticed that the number of sensitizing allergen molecules increased over time in cases. Furthermore, the range and the median number of molecules with IgE reactivity increased from EPA to 7 years. Interestingly, sensitization to each additional molecule from EPA to the age of 7 was significantly related to asthma at 7 years. Finally, sensitization to Bet v 1 and the number of sensitizing molecules at 7 years were significantly associated to asthma diagnosis at the same age.

The prevalence rates for allergic sensitization in the cases and controls at EPA are in the same range as in the previous population-based cohort studies in preschool age [33–35]. However, the asthma prevalence at the age of 7 years in our cohort is strikingly higher than previously reported in other cohorts of preschool wheezers [3, 36]. Nevertheless, the reported prevalence of asthma at 7 years in our study (68.1%) is as high as the ditto reported in a Dutch cohort of children with severe wheezing at early life (67%) [37]. The original GEWAC cohort is characterized by a high prevalence of recurrent wheezing (79%), and wheezing episodes that prompted inpatient care as well as hospitalization at inclusion (prevalence; 81%). These factors are recognized as predisposing for persistent asthma at school age [11, 38, 39]. Consequently, GEWAC can be considered as a high-risk cohort.

We found that food sensitization seems to dominate in EPA, whereas the prevalence of airborne sensitization increases markedly over time and surpasses the prevalence of food sensitization at school age. These observations are in line with other studies using IgE reactivity to a number of allergen extracts to define the allergic sensitization [14, 33].

Noteworthy, the prevalence and the type of sensitization as well as the asthma prevalence vary depending on socioeconomic status, extent of urbanization, ethnical origin, and geographic region [10, 13, 15, 40, 41]. Interestingly, not only the occurrence of allergic sensitization or asthma but also the strength of the association between these entities is shown to vary among different populations [13], which reflect the crucial role of genetics and environmental factors in atopy and asthma development.

Our main findings suggest that polysensitization, captured by molecular allergy diagnostics, has a crucial impact on both preschool wheezing and persistent asthma at school age, both cross-sectionally and longitudinally. Our results comply with previous reports regarding the

effect of early polysensitization on allergic multimorbidity including wheezing as early as at 2 years of age [42, 43]. Extract-based testing in cohorts of preschool wheezers has shown early polysensitization to be linked to persistent asthma at school age [42, 44, 45]. In addition, studies are pointing to the advantages of molecular allergy testing when assessing the allergic sensitization, particularly for polysensitized individuals [46] and the subsequent risk for atopic disease including asthma [25, 26, 47]. Our results regarding association between the median number of molecules with IgE reactivity at 7 years and asthma at the same age are in line with another cross-sectional study, which showed that children with asthma at 11 years were sensitized to significantly more allergen molecules at the same age than children without asthma [26]. Moreover, 2 cross-sectional Swedish studies underline the role of polysensitization by showing that the number of sensitizing dog allergen molecules was significantly related to the risk for reported asthma [48] and that sensitization to multiple lipocalin components was associated to severe asthma [49].

According to our results (online suppl. Table S2), no significant association between sensitization against any of the highest ranked molecules at EPA and asthma at 7 years was found. The age at inclusion in our study ranged from 6 months to 4 years with 36% (26/72) of cases being ≥ 24 months old when tested for allergic sensitization. Due to small sample of children being younger, the impact of sensitization below 2 years of age on later asthma development could not be clarified. Nevertheless, sensitization to Bet v 1 at 7 years was shown to be significantly associated to asthma at the same age (online suppl. Table S3). There is conflicting evidence about the role of sensitization to specific allergen molecules in asthma development. On the one hand, a study by Fontanella et al. [26] suggests that the pattern of interactions between molecule-specific IgE responses within each cluster of allergen molecules may predict asthma rather than the single molecule-specific IgE reactivity. On the other hand, several other studies have shown that sensitization to specific allergen molecules has a significant association as well as a predictive value for asthma [34, 50]. The identified “high-risk” allergen molecules comprise perennial allergens such as allergen molecules from pets [51], house dust mites [52], as well as pollens, and foods [34].

Using molecular diagnostics, we have demonstrated the development of molecular spreading among the children with a history of preschool wheezing in relation to the probability of persistent asthma at the age of 7. Howard et al. [18] have shown that the number of sensitizing

allergen molecules increases across different age points in childhood. However, it is still unclear how the molecular spreading influences the atopic march in the growing child. Longitudinal studies have shown that the initial IgE reactivity to a group of initiator allergen molecules may stay mono-molecular or expand to an oligo- or even poly-molecular stages [52, 53]. Our findings suggest that Bet v 1, Ara h 2, and Ara h 6, and Fel d 1 (cat) may have served as “initiator” molecules, triggering molecular spreading from EPA to school age and thereby affecting the development of persistent asthma at the age of 7 years.

Among the strengths of our study are the prospective design and the two-dimensional approach that allowed multiple comparisons between children suffering from acute wheeze and healthy controls, cross-sectionally and longitudinally. This approach enabled the assessment of allergic sensitization at different age points and its impact on two main clinical outcomes, preschool wheezing and persistent asthma at the age of 7. To our knowledge, this study is one of few studies which assess allergic sensitization longitudinally, from early childhood to school age, using analyses of 112 allergen molecules. Another asset was that the asthma diagnosis was according to the GINA guidelines and judged by the same pediatric allergologist. The included lung function measurements are objective parameters contributing to high internal validity.

Because of the size of our study population, we had limited statistical power to adjust for potential confounders or even detect effect modification by these factors. Our sample size was restricted by the availability of sera which potentially introduced selection bias. This is reflected in the age difference between the case and the control group, with the group of cases being slightly younger than the controls, which derived from the original age-matched groups. Furthermore, the measures of allergic sensitization in controls might be underestimated and the observed differences from the case group might be overestimated since known sensitization to airborne allergens at EPA was an exclusion criterion for the control group. Moreover, the use of standardized questionnaires filled out at the revisits to report symptoms and medication during a year may have introduced recall bias. As mentioned above, the features of the GEWAC cohort at inclusion indicate its high-risk nature which limits the external validity of this study. Nevertheless, zooming in the molecular sensitization profiles in a selected population with predominantly severe preschool wheeze makes this study unique in the field.

Conclusion

Polysensitization assessed on a molecular allergen level has a significant impact on both early-life wheezing and persistent asthma at school age. The notion of molecular spreading from preschool to school age is apparent and seems to contribute significantly to the risk for asthma at the age of 7 years in individuals with a history of wheezing in early life. Our results point to Bet v 1, Ara h 2, and Ara h 6, and Fel d 1 as potential markers of molecular spreading through which they may affect the course of asthma disease. Future research is warranted to shed light on “high risk” allergen molecules or sensitization profiles that may pave the way for novel prevention and therapeutic strategies.

Acknowledgments

We would like to thank all the participating children and their parents/legal guardians for making this study possible. We also thank Ann Berglind for valuable assistance in collecting data and biological sampling.

Statement of Ethics

This study has ethical approval from the Regional Ethics Committee at Karolinska Institutet, Stockholm (Dnr 2008/378-31/4 and Dnr 2014/399-31/3). Written and oral information about the study was provided to the parents, and written consent was obtained from parents and/or legal guardians prior to the study.

Conflict of Interest Statement

Anastasia Eleni Filiou, Idun Holmdahl, Anna Asarnej, Katarina Stenberg-Hammar, and Gunilla Hedlin have no conflicts of interest to declare. Marianne van Hage reports personal fees from Thermo Fisher Scientific, outside the submitted work. Niclas Rydell, Tina Ekencrantz, and Anders Sjölander are employees of Thermo Fisher Scientific. Dr Konradsen and Dr. Söderhäll report nonfinancial support from Thermo Fisher Scientific, during the conduct of the study.

Funding Sources

This work was financially supported by The Swedish Research Council, the Swedish Heart-Lung Foundation, Karolinska Institutet, Konsul Th C Bergh Foundation, Freemason Child House Foundation in Stockholm, the Centre for Allergy Research at Karolinska Institutet, the Samaritan Foundation, Sigurd and Elsa

Goljes Memorial Fund, Crown princess Lovisas association for Childcare, Axel Tielmans Memorial Fund, Region Stockholm (ALF project), the Cancer – and Allergy Foundation, The Swedish Asthma and Allergy Association's Research Foundation, the Hesselman Foundation, and King Gustaf V 80th Birthday Foundation. The funding sources had no influence on the study design, the collection, the analysis, the interpretation of the data, the writing of the manuscript, or the decision to submit the manuscript for publication.

Author Contributions

A.F., J.R.K., C.S., G.H., and K.S.-H. designed the study. A.F., J.R.K., and C.S. analyzed the data and performed statistical analyses. All authors were involved in data interpretation. A.F., J.R.K., and C.S. drafted the manuscript. All authors critically evaluated and revised the final manuscript.

Data Availability Statement

Due to the nature of this research, participants of this study did not agree for their data to be shared publicly, so supporting data is not available.

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