

ORIGINAL ARTICLE

WILEY

Single and multiple time-point allergic sensitization during childhood and risk of asthma by age 13

Ann-Marie Malby Schoos | Ea Jelding-Dannemand | Jakob Stokholm |
Klaus Bønnelykke | Hans Bisgaard  | Bo Lund Chawes 

COPSAC, Copenhagen Prospective Studies on Asthma in Childhood, Herlev and Gentofte Hospital, University of Copenhagen, Copenhagen, Denmark

Correspondence

Hans Bisgaard, COPSAC, Ledreborg Alle 34, DK-2820 Gentofte, Denmark.
Email: bisgaard@copsac.com

Funding information

All funding received by COPSAC is listed on www.copsac.com. The Lundbeck Foundation (Grant No. R16-A1694), the Ministry of Health (Grant No. 903516), Danish Council for Strategic Research (Grant No. 0603-00280B), and the Capital Region Research Foundation have provided core support to the COPSAC research center.

Edited by: Ömer Kalaycı

Abstract

Background: The relationship between allergic sensitization during childhood and risk of developing asthma remains unclear.

Objective: To analyze single time-point and temporal patterns of sensitization in childhood in relation to asthma at age 13.

Methods: Specific IgE (sIgE) level and skin prick test (SPT) toward 22 food allergens and aeroallergens were assessed at 6, 18 months, 4, 6, and 13 years in children from the high-risk Copenhagen Prospective Studies on Asthma in Childhood₂₀₀₀ (COPSAC₂₀₀₀) mother-child cohort. We analyzed the association between single time-point monosensitization, polysensitization, and quantitative assessment of sensitization, that is, sum of all sIgE levels and SPT wheal sizes, against asthma at age 13. In addition, we analyzed the association between three temporal patterns of sensitization: (a) early-transient, (b) late-onset, and (c) persistent sensitization and asthma.

Results: Polysensitization status measured by SPT or sIgE was at all single time-points associated with increased risk of asthma at age 13: OR range, SPT = 3.0-15.7, and sIgE = 2.6-15.7, respectively, whereas monosensitization status was inconsistently associated with asthma. Quantitative assessment of both sIgE and SPT results was associated with asthma at all single time-points: OR range, SPT = 1.3-3.6, and sIgE = 1.1-1.7. Persistent sensitization, but not early-transient or late-onset sensitization was associated with asthma by age 13: OR [95% CI], SPT = 8.9 [2.8-28.23], and sIgE = 2.9 [1.1-7.6], respectively.

Conclusion: Sensitization to multiple allergens at single time-points, increasing sIgE levels and SPT wheal sizes, and persistent sensitization during childhood were associated with increased risk of asthma at age 13, suggesting the use of quantitative and repetitive sensitization measurements when assessing risk of developing asthma.

KEYWORDS

asthma, children, monosensitization, patterns, polysensitization, sensitization, skin prick test, specific IgE

Abbreviations: CI, confidence interval; COPSAC, Copenhagen Prospective Studies on Asthma in Childhood; OR, odds ratio; sIgE, specific immunoglobulin E; SPT, skin prick test.

1 | INTRODUCTION

Assessment of allergic sensitization is used by physicians to diagnose food and inhalant allergies as part of the diagnosis and prediction of allergy-associated diseases such as asthma.¹ This approach is supported by a range of studies showing that food sensitization in early childhood (3–18 months) is a risk factor for later development of asthma.^{1–3} However, such approach is complicated by a poor and age-dependent agreement between sensitization determined by skin prick test (SPT) and specific immunoglobulin E (sIgE) levels,⁴ and by the fact that many children with sensitization are asymptomatic.^{4–6} Therefore, some guidelines have now excluded allergic sensitization assessments as part of the diagnosis of asthma,^{7,8} whereas some have kept it as part of the diagnostic criteria, but with low level of importance.⁹

We recently discovered and replicated seven latent patterns of sensitization during childhood using an unsupervised clustering analysis and showed that only the animal pattern (dog, cat, horse) was associated with asthma diagnosed based on quantification of symptoms and treatment response.¹⁰ These findings are in line with other attempts of asthma prediction from sensitization measurements using machine learning approaches (unsupervised, computerized pattern recognition),^{11–13} but given the complexity of these patterns, the findings are hard to implement in a clinical setting. Previous studies have found that specific temporal patterns of sensitization are associated with asthma,^{14–18} but there is a lack of agreement in classification of these patterns that complicates the clinical usefulness.

In this study, we analyzed measurements of sIgE and SPT results against 22 common food allergens and aeroallergens assessed longitudinally at ages 6, 18 months, 4, 6, and 13 years in children participating in the Copenhagen Prospective Studies on Asthma in Childhood 2000 (COPSAC₂₀₀₀) mother-child cohort. The aim was to investigate single time-point monosensitization (sensitization to only one allergen), polysensitization (sensitization to 2 or more allergens), and a quantitative assessment of degree of sensitization (sum of sIgE levels or SPT wheal sizes) at 6, 18 months, 4, 6, and 13 years and temporal patterns of sensitization (*early-transient sensitization*, defined as sensitized at 6 and/or 18 months and/or 4 and/or 6 years, but not sensitized at 13 years; *late-onset sensitization*, defined as sensitized at 13 years, but not earlier; and *persistent sensitization*, defined as both early sensitization and sensitization at 13 years of age) during childhood and their association with asthma at age 13.

2 | METHODS

2.1 | Design

Copenhagen Prospective Studies on Asthma in Childhood 2000 is a single-center, prospective clinical mother-child cohort study of 411 children born to mothers with a history of asthma between August 1998 and December 2001.^{19–21} The children were enrolled at age

Key Message

Children sensitized to multiple allergens and children with high levels of sIgE or large SPT wheal sizes at single time-points during childhood are at increased risk of asthma at age 13, which is also apparent for children with persistent sensitization, but not early-transient or late-onset sensitization during childhood.

1 month and subsequently followed with 6-monthly visits to the COPSAC research unit until the age of seven and again at 13 years of age. Additional acute care visits were arranged upon onset of asthma-, allergy-, and eczema-related symptoms.

2.2 | Ethics

The Copenhagen Ethics Committee (KF 01-289/96) and the Danish Data Protection Agency (2008-41-2434) approved the study, and oral and written informed consent was obtained from both parents at enrollment.

2.3 | Allergic sensitization

Skin prick test and sIgE measurements were performed at 6, 18 months, 4, 6, and 13 years of age against the following 12 aeroallergens: birch, grass, mugwort, horse, dog, cat, *D pteronyssinus*, *D farinae*, *Aspergillus fumigatus*, *Cladosporium herbarum*, *Penicillium notatum*, and *Alternaria alternata*, and the following 10 food allergens: milk, egg, wheat flour, soybean, peanut, cod, shrimp, rye flour, pork, and beef (Table S1).

Skin prick tests were performed using standard allergen extracts (ALK-Abelló, Soluprick® SQ, Hørsholm, Denmark). Histamine dihydrochloride (10 mg/mL) and physiological sodium chloride (9 mg/mL) were used as positive and negative controls, respectively. The average of the wheal diameter and its perpendicular was noted, and the test was considered positive if the wheal size was ≥ 2 mm greater than the negative control at ages 6 and 18 months and ≥ 3 mm at ages 4, 6, and 13 years⁴ (for further details, see the Online Repository).

Assessment of sIgE levels in blood samples was done by an initial screening method (ImmunoCAP Phadiatop Infant™ and ImmunoCAP Phadiatop™; Thermo Fisher Scientific, Uppsala, Sweden)²² followed by analysis of individual allergen sIgE levels in screening positive samples. Sensitization was defined as values of sIgE ≥ 0.35 kU_A/L.²¹

2.4 | Asthma

The diagnosis of asthma by age 13 was based on a previously detailed and validated quantitative algorithm based on symptoms recorded in daily diaries^{20,23} requiring all of the following: (a) recurrent episodes of troublesome lung symptoms (five episodes within

6 months, each lasting at least three consecutive days); (b) symptomatology typical of asthma, including exercise-induced symptoms, prolonged nocturnal cough, and persistent cough outside common cold; (c) need for intermittent rescue use of inhaled β_2 -agonist; and (d) response to a 3-month course of inhaled corticosteroids and relapse upon ended treatment.²⁰ The COPSAC study pediatricians solely did the diagnosis and treatment of asthma.

2.5 | Statistical analyses

The single time-point sensitization status at age 6, 18 months, 4, 6, and 13 years was analyzed as dichotomous variables (yes/no) as: *monosensitization*, defined by sensitization to only one allergen; *polysensitization*, defined by sensitization to more than one allergen; and also as a continuous variable: *quantitative degree of sensitization*, where the sum of sIgE levels and SPT wheal size results was analyzed based on a scale from 0 to 6 (see the Online Repository and Tables S2-S3 for details).

Allergic sensitization status was further analyzed as three temporal patterns: (a) *early-transient sensitization*, defined as sensitized at 6 and/or 18 months and/or 4 and/or 6 years, but not sensitized at 13 years; (b) *late-onset sensitization*, defined as sensitized at 13 years, but not earlier; and (c) *persistent sensitization*, defined as both early sensitization and sensitization at 13 years of age. *Never sensitized children* were defined as children with no positive test at any of the 5 time-points and were used as control group.

The association between the single time-point and temporal sensitization patterns and asthma at age 13 was analyzed using logistic regression with SPT and sIgE results analyzed separately. The results are presented as odds ratios (OR) with 95% CIs. The results are adjusted for father's asthma (all mothers have asthma), mother's elevated sIgE, father's elevated sIgE, and mother's smoking during 3rd trimester. All corresponding *P*-values were estimated by the Wald tests.

All statistical analyses were performed with SAS version 9.3 (SAS institute Inc).

P-values < .05 were considered statistically significant.

3 | RESULTS

3.1 | Baseline characteristics

Allergic sensitization status was assessed in 399 (97%) of the 411 children in the COPSAC₂₀₀₀ cohort at least once between the ages of 6 months and 13 years. By age 13, 40 children (12%) were diagnosed with asthma. Baseline characteristics including sex, social circumstances (see Table S4 for details), early-life environmental exposures, and allergic predisposition of the 399 children included in the study and the 12 excluded children, who were never tested for sensitization, are outlined in Table 1, which showed no differences between the groups. The prevalences of sensitization to the different allergens at the different time-points are depicted in Figure S1 (sIgE) and Figure S2 (SPT) in the Online Repository.

3.2 | Single time-point mono- and polysensitization and asthma at 13 years

3.2.1 | SPT monosensitization

Children with monosensitization at 6 months, 4, and 13 years had an increased risk of asthma at age 13: 6 months (monosensitization cases, *N* = 22), OR = 4.0 [1.5-10.7], *P* < .01; 4 years (*N* = 9), OR = 6.1 [1.6-24.1], *P* < .01; and 13 years (*N* = 41), OR = 2.4 [1.1-5.6], *P* = .04, but there were no significant associations between monosensitization at 18 months or 6 years and asthma: 18 months (*N* = 28), OR = 1.8 [0.6-5.1], *P* = .28; and 6 years (*N* = 22), OR = 1.8 [0.6-5.5], *P* = .34 (Table 2). When analyzing food allergens and aeroallergens separately, we found a consistent significant association between monosensitization to a food allergen at all ages and asthma at 13 years, and between monosensitization to an aeroallergen at 18 months, 4, and 13 years and asthma at 13 years (all *P* ≤ .03) (Table S5).

3.2.2 | SPT polysensitization

Only one child was polysensitized at age 6 months (who also had asthma at age 13 years), and the OR could therefore not be calculated for this time-point. At all other single time-points, polysensitization and asthma at 13 years were significantly associated and there appeared to be an age gradient; that is, the younger the child with polysensitization, the higher the risk of asthma: 18 months (polysensitization cases, *N* = 8), OR = 15.7 [2.8-89.0], *P* < .01; 4 years (*N* = 18), OR = 10.2 [3.6-28.8], *P* < .01; 6 years (*N* = 26), OR = 7.3 [2.9-18.3], *P* < .01; and 13 years (*N* = 71), OR = 3.0 [1.5-6.2], *P* < .01 (Table 2). When analyzing food allergens and aeroallergens separately, we also found a significant association at all time-points for both allergen groups where we had enough power to conduct an analysis (Table S6).

3.2.3 | sIgE monosensitization

There were no significant associations between monosensitization determined by sIgE at any single time-point and asthma at age 13: ORs range from 0.40 to 1.64 (Table 2). When analyzing food allergens and aeroallergens separately, we found a significant association between monosensitization to a food allergen at 13 years and to an aeroallergen at 18 months and asthma at 13 years (Table S5).

3.2.4 | sIgE polysensitization

There was a significant association between polysensitization at all time-points and asthma at 13 years: 6 months (polysensitization cases, *N* = 7), OR = 11.5 [2.5-53.5], *P* < .01; 18 months (*N* = 16), OR = 15.7 [4.8-51.1], *P* < .01; 4 years (*N* = 41), OR = 4.5 [2.0-10.0], *P* < .01; 6 years (*N* = 68), OR = 2.6 [1.3-5.6], *P* = .01; 13 years (*N* = 129), OR = 4.9 [2.3-10.5], *P* < .01 (Table 2), which also showed higher OR at younger ages similar to the SPT polysensitization

results. When analyzing food allergens and aeroallergens separately, we also found a significant association at all time-points for both allergen groups where we had enough power to conduct an analysis (Table S6).

3.3 | Single time-point quantitative sensitization analyses and asthma at 13 years

3.3.1 | SPT

We found that a higher SPT score at all single time-points among children with both mono- and polysensitization was significantly

associated with an increased risk of asthma at age 13: OR per score point at 6 months = 3.6 [1.5-8.3], $P < .01$; OR at 18 months = 1.5 [1.0-2.2], $P = .03$; OR at 4 years = 1.9 [1.4-2.6], $P < .01$; OR at 6 years = 1.6 [1.3-2.0], $P < .01$; OR at 13 years = 1.3 [1.2-1.5], $P < .01$.

3.3.2 | sIgE

Likewise, a higher sIgE score at all single time-points was significantly associated with asthma at 13 years: OR per score point at 6 months = 1.6 [1.2-2.2], $P < .01$; OR at 18 months = 1.7 [1.3-2.1], $P < .01$; OR at 4 years 1.2 [1.1-1.3], $P < .01$; OR at 6 years = 1.2

TABLE 1 Baseline characteristics of included children, who were tested for allergic sensitization at least once from 6 mo to 13 y, and excluded children, who were never tested

Characteristics	N (Total)	Included	Excluded	Included vs excluded
	411	399	12	<i>P</i> -value by chi-square test
Gender				
Male, N (%) ^a	411	199 (50%)	4 (33%)	.38
Socioeconomic factors				
Social circumstances, median (IQR) ^b	382	-0.090 (-0.747 to 0.585)	-0.838 (-1.165 to -0.511)	.18
Smoking during third trimester				
Yes, N (%) ^a	411	59 (15%)	4 (33%)	.10
Older siblings				
No older siblings, N (%)	401	242 (61%)	3 (100%) ^c	.59
1 older, N (%)		111 (28%)	0 (0%)	
2 older, N (%)		28 (7%)	0 (0%)	
≥3 older, N (%)		17 (4%)	0 (0%)	
Maternal allergic disposition				
Asthma: Yes, N (%)	411	399 (100%)	12 (100%)	NA
Allergic rhinitis: Yes, N (%)	410	330 (83%)	11 (92%)	.42
Eczema: Yes, N (%)	411	195 (49%)	6 (50%)	.94
Total IgE (kU _A /L) Median (IQR) ^b	352	52.8 (19.0 to 153.0)	NA ^d	NA ^d
Specific IgE ^e : Yes, N (%)	347	217 (63%)	NA ^d	NA ^d
Paternal allergic disposition				
Asthma: Yes, N (%)	399	68 (18%)	0 (0%)	.23
Allergic rhinitis: Yes, N (%)	397	128 (33%)	4 (33%)	1.00
Eczema: Yes, N (%)	397	52 (14%)	0 (0%)	.38
Total IgE (kU _A /L), median (IQR) ^b	317	35.0 (13.4 to 76.0)	NA ^d	NA ^d
Specific IgE ^e : Yes, N (%)	309	107 (35%)	NA ^d	NA ^d

^aFisher's exact test.

^bWilcoxon rank sum test.

^cOnly three of the non-tested children had data available on siblings.

^dNo available data on the non-tested children.

^ePositive sIgE ≥ 0.35 kU_A/L to any allergen.

TABLE 2 Association between monosensitization and polysensitization at different ages and asthma at 13 y of age

Age	Monosensitization					
	SPT			slgE		
	OR [95% CI]	P-value	N _{sensitized} /N _{total} ^a	OR [95% CI]	P-value	N _{sensitized} /N _{total} ^a
6 mo	4.02 [1.51-10.69]	.01	22/310	1.59 [0.44-5.80]	.48	21/314
18 mo	1.79 [0.63-5.07]	.28	28/298	1.64 [0.59-4.59]	.35	33/315
4 y	6.13 [1.56-24.11]	.01	9/269	0.93 [0.34-2.55]	.89	45/294
6 y	1.75 [0.55-5.53]	.34	22/270	0.40 [0.09-1.74]	.22	38/277
13 y	2.43 [1.05-5.61]	.04	42/312	0.68 [0.15-3.01]	.61	43/315
Age	Polysensitization					
	SPT			slgE		
	OR [95% CI] ^b	P-value	N _{sensitized} /N _{total} ^a	OR [95% CI] ^b	P-value	N _{sensitized} /N _{total} ^a
6 mo	NA ^b	NA ^b	1/310	11.46 [2.45-53.51]	<.01	7/314
18 mo	15.70 [2.77-89.04]	<.01	8/298	15.66 [4.80-51.11]	<.01	16/315
4 y	10.21 [3.62-28.83]	<.01	18/269	4.46 [2.00-9.96]	<.01	41/294
6 y	7.27 [2.89-18.29]	<.01	26/270	2.64 [1.25-5.58]	.01	68/277
13 y	3.03 [1.49-6.20]	<.01	69/312	4.88 [2.28-10.45]	<.01	101/315

^aChildren sensitized at the given age using the given method/total of children with complete data on sensitization and asthma.

^bThe OR could not be calculated as only one child was polysensitized at 6 mo.

Significant results are shown in bold.

[1.1-1.2], $P < .01$; and OR at 13 years = 1.1 [1.1-1.2], $P < .01$ (Table 3).

3.4 | Temporal sensitization patterns and asthma at 13 years

3.4.1 | SPT

Among the sensitization patterns, persistent sensitization ($N = 33$) was a significant risk factor for asthma at 13 years: OR = 8.9 [2.8-28.3], $P < .01$, whereas no significant associations were detected between early-transient sensitization ($N = 11$) or late-onset sensitization ($N = 33$) and asthma: OR = 4.0 [0.7-23.4], $P = .13$ and OR = 2.5 [0.6-9.8], $P = .20$, respectively (Table S7).

3.4.2 | slgE

Specific IgE analyses also showed that persistent sensitization ($N = 75$) was the only pattern significantly associated with asthma at 13 years: OR = 2.9 [1.1-7.6], $P = .03$. There was no significant association between late-onset sensitization ($N = 27$) and asthma: OR = 2.9 [0.8-9.9], $P = .10$, whereas no results could be computed for early-transient sensitization ($N = 18$), as none of these children had asthma at 13 years of age (Table S7). The results of the SPT and slgE temporal sensitization pattern analyses are summarized in Figure 1.

Sensitivity analyses adjusting all of the models for father's asthma (all mothers have asthma), mother's elevated slgE, father's elevated slgE, and mother's smoking during 3rd trimester did not alter the results noteworthy (Tables S8-S10).

TABLE 3 Association between the quantitative sensitization score at different ages and asthma at 13 y of age

Age	SPT			slgE		
	OR [95% CI] ^a	P-value	N _{sensitized} /N _{total} ^b	OR [95% CI] ^a	P-value	N _{sensitized} /N _{total} ^b
6 mo	3.58 [1.54-8.33]	<.01	23/335	1.63 [1.23-2.17]	<.01	25/314
18 mo	1.51 [1.04-2.20]	.03	27/320	1.67 [1.33-2.10]	<.01	42/314
4 y	1.88 [1.39-2.56]	<.01	27/281	1.19 [1.09-1.30]	<.01	81/294
6 y	1.57 [1.26-1.97]	<.01	40/272	1.15 [1.08-1.23]	<.01	93/277
13 y	1.34 [1.18-1.52]	<.01	110/338	1.11 [1.06-1.15]	<.01	146/315

^aLogistic regression was used in the analyses calculating the OR of asthma at 13 y each time the sensitization score is increased by 1.

^bChildren sensitized at the given age using the given method/total of children with complete data on sensitization and asthma.

Significant results are shown in bold.

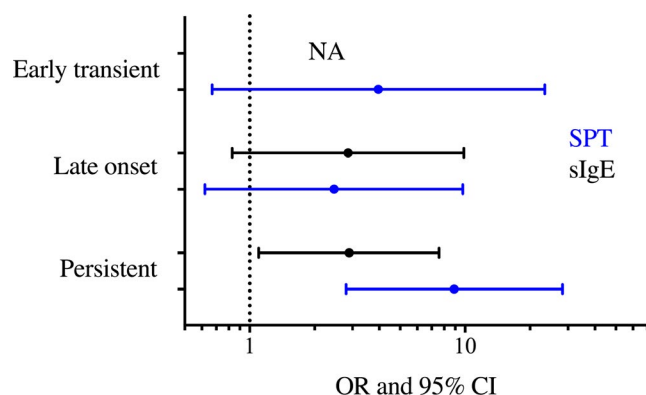


FIGURE 1 Association between temporal sensitization patterns and asthma at 13 y of age. None of the children classified as early-transient sIgE ($N = 18$) had asthma at 13 y, and the OR could therefore not be calculated. Sensitization SPT $N/N_{\text{total}} = 77/171$. Sensitization sIgE $N/N_{\text{total}} = 120/215$ [Colour figure can be viewed at wileyonlinelibrary.com]

4 | DISCUSSION

4.1 | Primary findings

Polysensitization status measured repetitively since infancy at single time-points by SPT or sIgE was associated with increased risk of asthma at age 13, whereas monosensitization status was inconsistently associated with asthma. Single time-point quantitative assessments of both sIgE and SPT results were associated with asthma at all time-points in both mono- and polysensitized children, suggesting the use of quantitative over dichotomized assessment. Persistent sensitization, but not early-transient or late-onset sensitization was associated with asthma by age 13, which underscores the importance of repetitive sensitization assessments in children with no sensitization or monosensitization when assessing risk of developing asthma.

4.2 | Strengths and limitations of the study

The prospective birth cohort design with the longitudinal repetitive measurements of sensitization by both SPT and sIgE levels at five time-points through childhood is a major strength of this study. This ensured that temporal patterns could be defined and that all analyses could be done with sensitization status by SPT and sIgE separately, which is important as there is a poor overlap between SPT and sIgE results in early childhood.⁴

All SPTs were performed by the same trained research personnel, results were double-checked and locked in a database, and the asthma diagnosis was obtained solely by the COPSAC pediatricians based on a pre-defined, validated algorithm, which limited the risk of assessment bias.^{20,21} Furthermore, the diagnostic criteria for asthma did not include information on allergic sensitization, thus allowing assessment of the association between sensitization and asthma without bias from the common belief of a relationship between these conditions.

The study participants included children at increased risk of asthma, which may limit the generalization of our findings. Few

children were polysensitized at age 6 months, especially diagnosed by SPT ($N = 1$), which precluded investigation of association with asthma. However, we observed that the quantitative sensitization scores at age 6 months derived from both SPT and sIgE results were significantly associated with asthma development. There were also few children with early-transient sensitization ($N = 11$) when diagnosed by SPT, and the result of no association with asthma at age 13 should therefore be interpreted with caution. When diagnosed by sIgE, none of the children with early-transient sensitization ($N = 18$) had asthma, which means no OR could be calculated; however, it supports the SPT findings of no association with asthma.

4.3 | Interpretation

Sensitization to multiple allergens at single time-points, quantitative assessment of sensitization status, and persistent sensitization during childhood were associated with increased risk of asthma at age 13, suggesting the use of quantitative sensitization scores and repetitive sensitization measurements when assessing risk of developing asthma. Furthermore, there appeared to be an age gradient in polysensitized children, especially diagnosed by SPT; that is, the younger the child with polysensitization, the higher the risk of asthma.

Our findings align with a recent study of 1056 children demonstrating that children with polysensitization (food allergens and/or aeroallergens) at 18 months of age compared to monosensitized children were more likely to have current symptoms of asthma at age 6.²⁴ Further, a study of 714 children showed that the cumulative prevalence of asthma till age 13 increased with the number of positive SPTs to aeroallergens²⁵ and another study of 289 children showed that aeroallergen polysensitization status at 1, 3, and 6 years was significantly associated with asthma at 6-8 years.²⁶ In addition, a birth cohort study of 273 children found an increased risk of asthma at 13 years in children with sIgE sensitization to aeroallergens before the age of 2 years,¹⁵ which was stronger among children who developed polysensitization.¹⁵ Contrastingly, results from the GINIplus study of 1290 children showed that sIgE sensitization at age 1 year (food allergen and/or aeroallergen) increased the risk of asthma at 6 years, but without an added risk from polysensitization.²⁷ Together, there is an overweight of studies showing association between polysensitization status and asthma, but the data are ambiguous. Importantly, the indiscriminate use of SPT and sIgE as markers of sensitization may explain the diversity in the study findings, as these tests are highly incongruent, particularly in early life.⁴ Our study is the only one to include longitudinal measurements of both SPT and sIgE in the same cohort. Furthermore, when looking at SPT monosensitization, the type of allergen, that is, food allergen or aeroallergen, seemed to be important. Surprisingly, we found that the association between food allergen monosensitization and asthma at 13 years was more consistent over time than between aeroallergen monosensitization and asthma at 13 years. We chose the low sIgE cutoff of 0.35 kU_A/L for our analyses, as we wanted to investigate sensitization as a biomarker for later development of asthma, and not a clinically relevant allergy.

The consistent association between single time-point polysensitization and risk of asthma that we observed may be explained by the phenomenon called “molecular spreading,” which is believed to origin upon extensive exposure to an allergenic source.²⁸ Thus, previous studies utilizing component-resolved diagnostics have shown that polysensitization to allergen components, that is, an epitope-spreading process of the IgE response, precedes the development of clinical disease²⁸ and that polysensitization to lipocalins (the largest mammalian allergen family) associates with more severe asthma.²⁹

We also showed a consistent association between the quantitative sensitization score and risk of asthma, meaning that the higher the level of all sIgE levels or the bigger the SPT total wheal sizes, the higher the risk of getting asthma at 13. This association was significant at all time-points and demonstrates a dose-response relationship in support of a true association between degree of sensitization, polysensitization, persistent sensitization, and asthma.

Our analysis of temporal patterns of sensitization through childhood showed for both SPT and sIgE results that children with persistent sensitization, but not early-transient or late-onset sensitization were at increased risk of asthma by age 13. These findings underscore the clinical importance of conducting repetitive sensitization assessments during childhood to determine a child's risk of developing asthma.

In line with our findings, a study of 1314 unselected children showed no increased risk of asthma at 7 years among children with early-transient sensitization, defined as a positive sIgE to any allergen before 2 years of age.¹⁴ Similar results of association between persistent sensitization and asthma, but not early-transient sensitization, were shown in another unselected cohort study of 1536 children, where the children were followed from age 1 to 10 years, defining persistent sensitization as a positive SPT toward inhalant allergens at the ages 4 and 10 years and early-transient sensitization as a positive SPT only at 4 years.¹⁸ Together, these studies and our findings suggest that persistent sensitization is of importance when assessing risk of developing asthma, keeping in mind that the definition of persistent sensitization varies from study to study and that our study is the first to include longitudinal data on both SPT and sIgE measurements in the same cohort.

Thus far, all previous studies have shown that isolated late-onset sensitization is not a risk factor for asthma.^{14,15,17,18} We have previously shown an association between any sensitization at 13 years and current asthma,³⁰ but in this study, we found no association between late-onset sensitization (sensitization only at 13 years) and current asthma, indicating that the persistent sensitization group is driving the former finding. It should, however, be noted that we found a borderline significant association between late-onset sensitization diagnosed by sIgE and asthma ($P = .10$), and the result should be interpreted with caution as the low number of cases with late-onset sensitization ($N = 27$) may mask a true association (type II error).

The lack of or sparse association between monosensitization at single time-points and isolated early-transient and late-onset sensitization and asthma by age 13 may be an indication of a healthier confined immune response to allergen exposure and a subsequent phenotype with a lower risk of asthma.³¹ Correspondingly, the increased risk of asthma

among children with polysensitization at single time-points, the quantitative increasing risk of asthma with increasing levels of sIgE and SPT wheal sizes, and increased asthma risk among children with persistent sensitization may indicate a phenotype with a more unhealthy and aggressive immune response upon exposure to allergens.

5 | CONCLUSION

This study provides new insight for the clinicians into the interpretation of single time-point evaluation and multiple time-point evaluation of allergic sensitization to determine a child's risk of developing asthma. For single time-point assessments, children sensitized to multiple allergens and particularly children with high levels of sIgE or large SPT wheal sizes are at increased risk of asthma, which is also apparent for children with persistent sensitization during childhood, but not for children with early-transient or late-onset sensitization.

ACKNOWLEDGMENTS

We gratefully express our gratitude to the children and families of the COPSAC cohort study for all their support and commitment. We acknowledge and appreciate the unique efforts of the COPSAC research team.

CONFLICT OF INTEREST

The authors have no conflicts of interest relevant to this topic. The corresponding author had full access to the data and had final responsibility for the decision to submit for publication.

AUTHOR CONTRIBUTIONS

HB is the guarantor of the study and has contributed to the conception and design to conduct of the study and acquisition of data, data analysis, and interpretation of data. AMS and EJD have contributed equally to the manuscript. All co-authors have provided important intellectual input and contributed considerably to the analyses and interpretation of the data. All authors guarantee that the accuracy and integrity of any part of the work has been appropriately investigated and resolved and approved the final version of the manuscript. The corresponding author had full access to the data and had final responsibility for the decision to submit for publication. No honorarium, grant, or other form of payment was given to any of the authors to produce this manuscript.

GOVERNANCE

We are aware of and comply with recognized codes of good research practice, including the Danish Code of Conduct for Research Integrity. We comply with national and international rules on the safety and rights of patients and healthy subjects, including Good Clinical Practice (GCP) as defined in the EU's Directive on Good Clinical Practice, the International Conference on Harmonisation's (ICH)

good clinical practice guidelines, and the Helsinki Declaration. We follow national and international rules on the processing of personal data, including the Danish Act on Processing of Personal Data and the practice of the Danish Data Inspectorate.

ORCID

Hans Bisgaard  <https://orcid.org/0000-0003-4131-7592>

Bo Lund Chawes  <https://orcid.org/0000-0001-6846-6243>

REFERENCES

- Høst A, Andrae S, Charkin S, et al. Allergy testing in children: why, who, when and how? *Allergy*. 2003;58:559-569.
- Kjaer HF, Eller E, Andersen KE, Høst A, Bindslev-Jensen C. The association between early sensitization patterns and subsequent allergic disease. The DARC birth cohort study. *Pediatr Allergy Immunol*. 2009;20:726-734.
- Rhodes HL, Sporik R, Thomas P, Holgate ST, Cogswell JJ. Early life risk factors for adult asthma: a birth cohort study of subjects at risk. *J Allergy Clin Immunol*. 2001;108:720-725.
- Schoos A-MM, Chawes BL, Følsgaard NV, Samandari N, Bønnelykke K, Bisgaard H. Disagreement between skin prick test and specific IgE in young children. *Allergy*. 2015;70:41-48.
- Pallasaho P, Rönmark E, Haahtela T, Sovijärvi A, Lundbäck B. Degree and clinical relevance of sensitization to common allergens among adults: a population study in Helsinki. *Finland Clin Exp Allergy*. 2006;36:503-509.
- Bousquet J, Anto JM, Wickman M, et al. Are allergic multimorbidities and IgE polysensitization associated with the persistence or re-occurrence of foetal type 2 signalling? The MeDALL hypothesis. *Allergy*. 2015;70:1062-1078.
- <https://www.brit-thoracic.org.uk/qualityimprovement/guidelines/asthma/> (accessed 21 Jul2019).
- asthma-2098547456965.
- Papadopoulos NG, Arakawa H, Carlsen KH, et al. International consensus on (ICON) pediatric asthma. *Allergy*. 2012;67:976-997.
- Schoos A-MM, Chawes BL, Melén E, et al. Sensitization trajectories in childhood revealed by using a cluster analysis. *J Allergy Clin Immunol*. 2017;140:1693-1699.
- Simpson A, Tan VY, Winn J, et al. Beyond atopy: multiple patterns of sensitization in relation to asthma in a birth cohort study. *Am J Respir Crit Care Med*. 2010;181:1200-1206.
- Lazic N, Roberts G, Custovic A, et al. Multiple atopy phenotypes and their associations with asthma: similar findings from two birth cohorts. *Allergy*. 2013;68:764-770.
- Schmitz R, Ellert U, Kalckloesch M, Dahm S, Thamm M. Patterns of sensitization to inhalant and food allergens-findings from the German Health Interview and Examination Survey for Children and Adolescents. *Int Arch Allergy Immunol*. 2013;162:263-270.
- Illi S, von Mutius E, Lau S, et al. The pattern of atopic sensitization is associated with the development of asthma in childhood. *J Allergy Clin Immunol*. 2001;108:709-714.
- Matricardi PM, Bockelbrink A, Keil T, et al. Dynamic evolution of serum immunoglobulin E to airborne allergens throughout childhood: results from the Multi-Centre Allergy Study birth cohort. *Clin Exp Allergy*. 2009;39:1551-1557.
- Sherrill D, Stein R, Kurzius-Spencer M, Martinez F. On early sensitization to allergens and development of respiratory symptoms. *Clin Exp Allergy*. 1999;29:905-911.
- Peat JK, Salome CM, Woolcock AJ. Longitudinal changes in atopy during a 4-year period: Relation to bronchial hyperresponsiveness and respiratory symptoms in a population sample of Australian schoolchildren. *J Allergy Clin Immunol*. 1990;85:65-74.
- Kurukulaaratchy RJ, Matthews S, Arshad SH. Defining childhood atopic phenotypes to investigate the association of atopic sensitization with allergic disease. *Allergy*. 2005;60:1280-1286.
- Bisgaard HT. The Copenhagen Prospective Study on Asthma in Childhood (COPSAC): design, rationale, and baseline data from a longitudinal birth cohort study. *Ann Allergy Asthma Immunol*. 2004;93:381-389.
- Bisgaard H, Hermansen MN, Loland L, Halkjaer LB, Buchvald F. Intermittent inhaled corticosteroids in infants with episodic wheezing. *N Engl J Med*. 2006;354:1998-2005.
- Bisgaard H, Hermansen MN, Buchvald F, et al. Childhood asthma after bacterial colonization of the airway in neonates. *N Engl J Med*. 2007;357:1487-1495.
- Paganelli R, Ansotegui IJ, Sastre J, et al. Specific IgE antibodies in the diagnosis of atopic disease. Clinical evaluation of a new in vitro test system, UniCAP, in six European allergy clinics. *Allergy*. 1998;53:763-768.
- Bisgaard H, Bønnelykke K, Sleiman PM, et al. Chromosome 17q21 gene variants are associated with asthma and exacerbations but not atopy in early childhood. *Am J Respir Crit Care Med*. 2009;179:179-185.
- Gabet SE, Just J, Couderc R, Bousquet J, Seta N, Momas I. Early polysensitization is associated with allergic multimorbidity in PARIS birth cohort infants. *Pediatr Allergy Immunol*. 2016;27:831-837.
- Sears MR, Herbison GP, Holdaway MD, Hewitt CJ, Flannery EM, Silva PA. The relative risks of sensitivity to grass pollen, house dust mite and cat dander in the development of childhood asthma. *Clin Exp Allergy*. 1989;19:419-424.
- Stoltz DJ, Jackson DJ, Evans MD, et al. Specific patterns of allergic sensitization in early childhood and asthma & rhinitis risk. *Clin Exp Allergy*. 2013;43:233-241.
- Brockow I, Zutavern A, Hoffmann U, et al. Early allergic sensitizations and their relevance to atopic diseases in children aged 6 years: results of the GINI study. *J Invest Allergol Clin Immunol*. 2009;19:180-187.
- Hatzler L, Panetta V, Lau S, et al. Molecular spreading and predictive value of preclinical IgE response to *Phleum pratense* in children with hay fever. *J Allergy Clin Immunol*. 2012;130:894-901.e5.
- Konradsen JR, Nordlund B, Onell A, Borres MP, Grönlund H, Hedlin G. Severe childhood asthma and allergy to furry animals: refined assessment using molecular-based allergy diagnostics. *Pediatr Allergy Immunol*. 2014;25:187-192.
- Schoos A-MM, Chawes BL, Rasmussen MA, Bloch J, Bønnelykke K, Bisgaard H. Atopic endotype in childhood. *J Allergy Clin Immunol*. 2016;137:844-851.e4.
- Kim KW, Kim EA, Kwon BC, et al. Comparison of allergic indices in monosensitized and polysensitized patients with childhood asthma. *J Korean Med Sci*. 2006;21:1012-1016.

SUPPORTING INFORMATION

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

How to cite this article: Schoos A-MM, Jelding-Dannemand E, Stokholm J, Bønnelykke K, Bisgaard H, Chawes BL. Single and multiple time-point allergic sensitization during childhood and risk of asthma by age 13. *Pediatr Allergy Immunol*. 2019;30:716-723. <https://doi.org/10.1111/pai.13109>

1 **Appendix – online repository:**

2

3

4 **Methods**

5 *SPT*: All tests were done by the same experienced research personnel placing a drop of each
6 allergen extract and controls on the children's volar forearm and using a sterile lancet to prick
7 approximately 1 mm through the cutis. The reaction to the positive control was read after 10 min,
8 whereas the reactions to the negative control and the allergen extracts were read after 15 min. The
9 children were informed not to use anti-histamines within 72 hours of testing (the positive control
10 was used as screening for use of antihistamines with longer half-life) as well as application of mild
11 steroid creams (group 1-2) on the arms 24 hours before testing. Stronger steroid creams (group 3-4)
12 were not used in our cohort⁴.

13 The *quantitative degree of sensitization* was based on the sum of sIgE levels and SPT wheal size
14 results and was analyzed using a scale from 0 to 6. For sIgE, level 0 = sIgE<0.35 kU_A/L, level
15 1=0.35-0.69 kU_A/L, level 2=0.70-3.49 kU_A/L, level 3=3.50-17.49 kU_A/L, level 4=17.50-49.99
16 kU_A/L, level 5=50.00-100.00 kU_A/L, level 6= sIgE>100.00 kU_A/L (**Tables S2**). For SPT, level 0 =
17 wheal size<2 mm (½ and 1½ years) and <3mm (4, 6, and 13 years), level 1=2-4 mm (½ and 1½
18 years) and 3-4 mm (4, 6, and 13 years), level 2=5-6 mm, level 3=7-8 mm, level 4=9-10 mm, level
19 5=11-12 mm, level 6 = wheal size 13 mm and above (**Table S3**). For the quantitative analysis, only
20 allergens tested at all five time points by both SPT and sIgE, were included in the analyses (**Table**
21 **S1**).

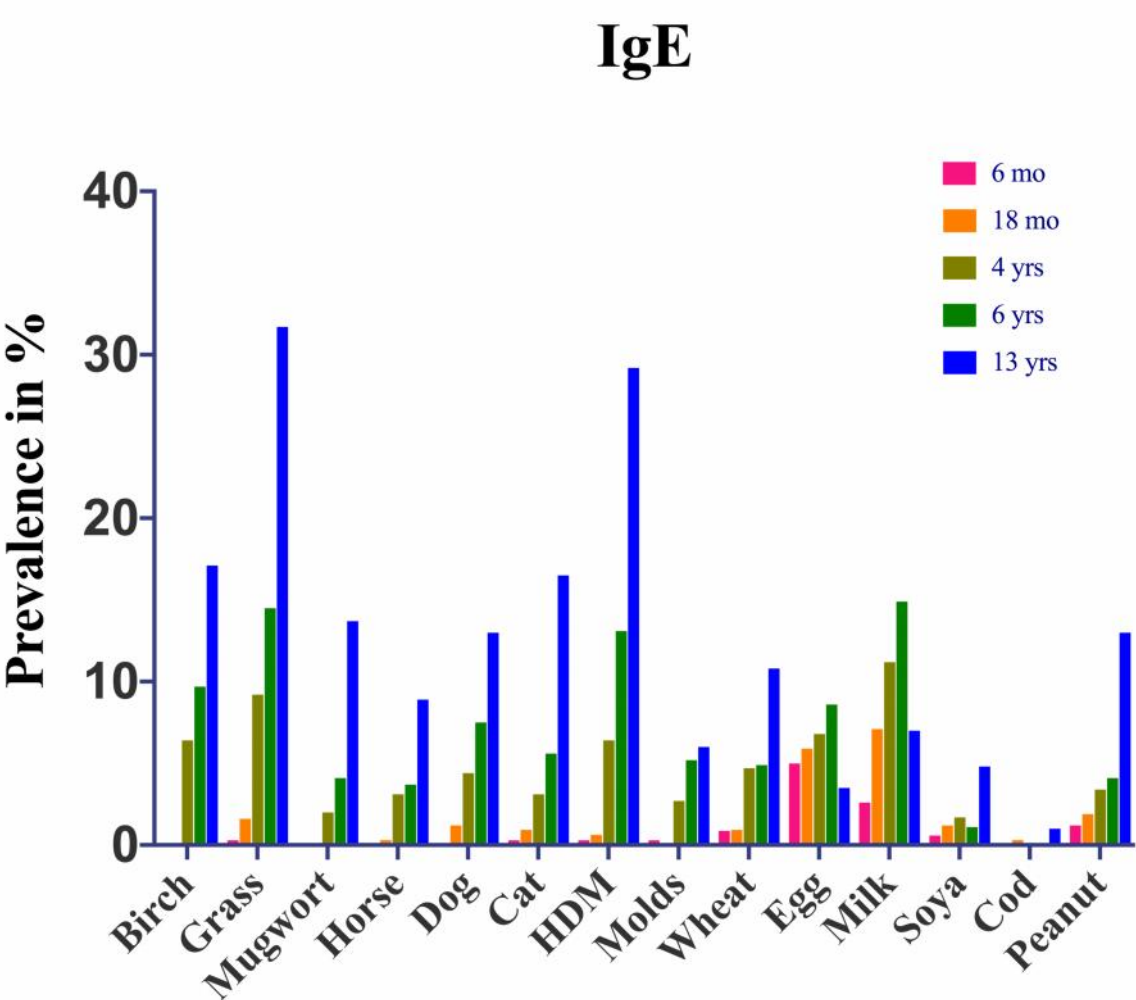
22

23

24

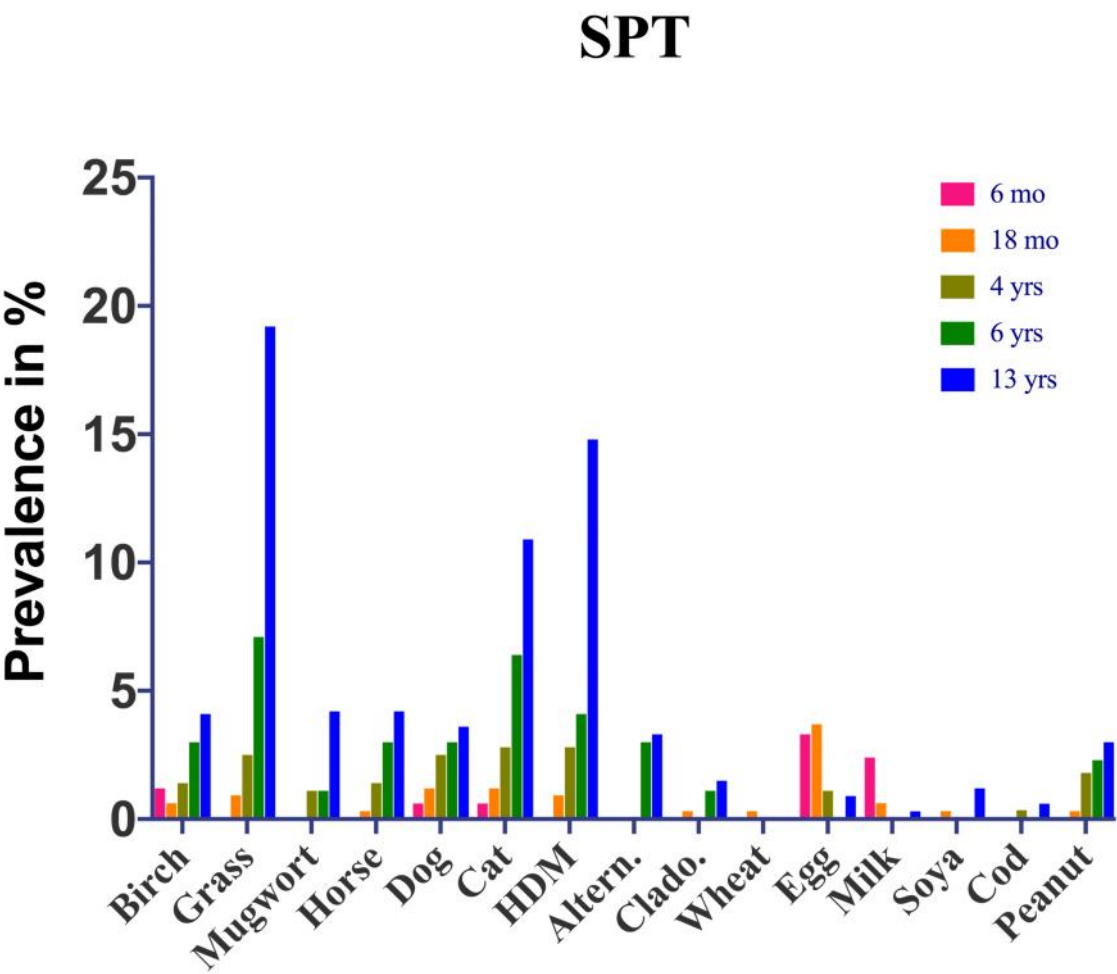
25 Figures

26
27 **Figure S1: Prevalence of sensitization to the different allergens and the different time points**
28 **measured by sIgE**
29



30
31
32 **HDM: house dust mite *D. pteronyssinus***
33

34 Figure S2: Prevalence of sensitization to the different allergens and the different time points
35 measured by SPT
36



37
38 HDM: house dust mite *D. pteronyssinus*
39 Altern.: *Alternaria alternate*
40 Cladospo.: *Cladosporium herbarum*

41 **Table S1: Allergens tested by SPT and/or sIgE in the COPSAC₂₀₀₀ birth cohort. Allergens in**
42 **bold font have been tested by both SPT and sIgE.**
43

Inhalant	Food
Common silver birch	Milk
Timothy grass	Egg
Mugwort	Wheat flour
Horse	Soybean
Dog	Peanut
Cat	Cod
D. pteronyssinus (house dust mite)	Shrimp
D. farinae (house dust mite)	Rye flour
Aspergillus fumigatus (mold)	Pork
Cladosporium herbarum (mold)	Beef
Penicillium notatum (mold)	
Alternaria alternata(mold)	

44
45
46

47 **Table S2: sIgE score**

RAST score	IgE level (kU/l)
0	< 0.35
1	0.35 – 0.69
2	0.70 – 3.49
3	3.50 – 17.49
4	17.50 – 49.99
5	50.00 – 100.00
6	> 100.00

48
49
50
51
52
53
54

55 **Table S3: SPT score**

SPT rating	SPT wheal size diameter in mm
0	< 2 (6 + 18 mth) < 3 (4-13 yrs)
1	2(6 + 18 mth) /3(4-13 yrs) - 4
2	5-6
3	7-8
4	9-10
5	11-12
6	13 and over

56

57

58 **Table S4: Baseline table of original information used to derive the measure of social**
59 **circumstances in Copsac2000**

	Variable type	categorical: n (%) numeric: mean (SD)	Missing data
Household income	<i>cat</i>		29
Below 400.00		120 (31.41)	
400.000-600.000		153 (40.05)	
600.000-800.000		76 (19.90)	
800.000-1.000.000		22 (5.76)	
Above 1.000.000		11 (2.88)	
Maternal education	<i>cat</i>		29
Elementary or College		220 (57.59)	
Medium		104 (27.23)	
University		58 (15.18)	
Maternal age	<i>num</i>	32.11 (4.51)	0

Social circumstances are correlated to household income ($r=0.73$, $p<0.001$), to maternal level of education ($r=0.68$, $p<0.001$) and to maternal age ($r=0.75$, $p<0.001$).

60

61

62 **Table S5:** Association between monosensitization to food- and aeroallergens at different ages and
63 asthma at 13 years of age.
64

Age	Monosensitization for food allergens					
	SPT			sIgE		
	OR [95% CI]	P-value	Nsensi/N [§]	OR [95% CI]	P-value	Nsensi/ N [§]
6 mo	3.56 [1.18-10.99]	0.03	16/311	1.98 [0.54-7.32]	0.30	17/311
18 mo	3.45 [1.14-10.45]	0.03	17/298	1.26 [0.35-4.48]	0.72	26/315
4 yrs	6.98 [1.76-27.68]	0.01	9/254	2.41 [0.90-6.51]	0.08	29/294
6 yrs	14.18 [3.21-62.69]	<0.01	8/265	1.33 [0.47-3.74]	0.60	37/277
13 yrs	5.15 [1.17-22.59]	0.03	8/310	7.03 [2.74-18.08]	<0.01	22/315

Age	Monosensitization for aeroallergens					
	SPT			sIgE		
	OR [95% CI]*	P-value	Nsensi/ N [§]	OR [95% CI]*	P-value	Nsensi/N [§]
6 mo	3.72 [0.66-21.05]	0.14	6/311	NA	NA	2/313
18 mo	3.55 [1.16-10.89]	0.03	16/298	22.83 [5.59-93.27]	<0.01	10/313
4 yrs	12.22 [3.45-43.29]	<0.01	11/269	2.01 [0.70-5.77]	0.20	31/294
6 yrs	2.31 [0.71-7.47]	0.16	23/270	0.63 [0.14-2.82]	0.54	32/277
13 yrs	2.97 [1.15-7.64]	0.02	47/312	2.09 [0.60-7.29]	0.25	43/315

[§] Total of children with complete data on sensitization and asthma

67 Table S6: Association between polysensitization to food- and aeroallergens at different ages and
68 asthma at 13 years of age.
69

Age	Polysensitization for food allergens					
	SPT			sIgE		
	OR [95% CI]	P-value	Nsensi/N [§]	OR [95% CI]	P-value	Nsensi/ N [§]
6 mo	NA	NA	1/311	4.06 [1.19-13.91]	<0.01	7/311
18 mo	16.53 [1.46-187.83]	0.02	3/298	13.51 [4.00-45.62]	<0.01	12/315
4 yrs	NA	NA	2/254	4.27 [1.49-12.26]	<0.01	19/294
6 yrs	NA	NA	0/265	3.18 [1.13-8.93]	0.03	22/277
13 yrs	12.87 [2.07-80.03]	<0.01	5/310	4.06 [1.19-13.91]	0.03	14/315
Age	Polysensitization for aeroallergens					
	SPT			sIgE		
	OR [95% CI]*	P-value	Nsensi/ N [§]	OR [95% CI]*	P-value	Nsensi/ N [§]
6 mo	NA	NA	1/311	NA	NA	0/313
18 mo	NA	NA	0/298	NA	NA	1/313
4 yrs	10.18 [3.03-34.26]	<0.01	12/269	8.02 [3.14-20.50]	<0.01	23/294
6 yrs	8.96 [3.30-24.31]	<0.01	20/270	4.51 [1.97-10.33]	<0.01	37/277
13 yrs	4.82 [2.17-10.70]	<0.01	64/312	7.06 [3.06-16.33]	<0.01	101/315

[§] Total of children with complete data on sensitization and asthma

72 Table S7: Association between temporal sensitization patterns and asthma at 13 years of age.

Sensitization pattern	SPT			sIgE		
	OR [95% CI]*	P-value	Nsensi/Ntotal	OR [95% CI]*	P-value	Nsensi/Ntotal
Early transient	3.96 [0.67-23.39]	0.13	11/171	NA	NA	18/215
Late-onset	2.46 [0.62-9.76]	0.20	33/171	2.86 [0.83-9.87]	0.10	27/215
Persistent	8.90 [2.80-28.26]	<0.01	33/171	2.89 [1.10-7.57]	0.03	75/215

Complete data on SPT and asthma were available on 171 children; 22 had asthma.

Complete data on sIgE and asthma were available on 215 children; 26 had asthma

*never sensitized children used as control group

73

74

75 **Table S8:** Association between monosensitization and polysensitization at different ages and
 76 asthma at 13 years of age adjusted for father’s asthma (all mothers have asthma), mother’s elevated
 77 sIgE, father’s elevated sIgE and mother’s smoking during 3rd trimester.
 78

Age	Monosensitization					
	SPT			sIgE		
	aOR [95% CI]	P-value	Nsensi/N [§]	aOR [95% CI]	P-value	Nsensi/ N [§]
6 mo	2.94 [0.94-9.22]	0.06	19/259	1.25 [0.25-6.09]	0.79	15/261
18 mo	2.37 [0.76-7.41]	0.07	23/258	1.90 [0.50-7.25]	0.35	21/271
4 yrs	10.99 [2.40-50.36]	<0.01	8/249	1.12 [0.29-4.23]	0.87	35/270
6 yrs	2.28 [0.56-9.28]	0.25	19/253	0.51 [0.11-2.40]	0.40	38/255
13 yrs	2.10 [0.71-6.26]	0.18	42/312	1.94 [0.46-8.19]	0.37	32/264
Age	Polysensitization					
	SPT			sIgE		
	aOR [95% CI]*	P-value	Nsensi/ N [§]	aOR [95% CI]*	P-value	Nsensi/N [§]
6 mo	NA	NA	1/259	11.91 [2.36-59.97]	<0.01	7/261
18 mo	41.13 [4.13-409.87]	<0.01	5/258	25.68 [6.67-98.83]	<0.01	12/271
4 yrs	15.67 [4.93-49.67]	<0.01	16/249	5.86 [2.43-14.13]	<0.01	40/270
6 yrs	11.71 [3.95-34.70]	<0.01	20/253	3.35 [1.38-8.11]	<0.01	48/255
13 yrs	4.23 [1.78-10.08]	<0.01	69/312	6.67 [2.63-16.96]	<0.01	87/264

§ Total of children with complete data on sensitization and asthma

81 **Table S9:** Association between the quantitative sensitization score at different ages and asthma at
 82 13 years of age adjusted for father’s asthma (all mothers have asthma), mother’s elevated sIgE,
 83 father’s elevated sIgE and mother’s smoking during 3rd trimester.
 84

Age	SPT			sIgE		
	aOR [95% CI]*	P-value	Nsensi/ N [§]	aOR [95% CI]*	P-value	Nsensi/ N [§]
6 mo	2.84 [1.23-6.57]	0.01	19/280	1.74 [1.24-2.44]	<0.01	22/261
18 mo	2.07 [1.06-4.05]	0.03	22/277	2.18 [1.52-3.11]	<0.01	32/270
4 yrs	2.14 [1.51-3.03]	<0.01	22/260	1.21 [1.09-1.35]	<0.01	74/270
6 yrs	1.53 [1.22-1.93]	<0.01	34/255	1.19 [1.10-1.28]	<0.01	84/255
13 yrs	1.33 [1.16-1.53]	<0.01	91/280	1.11 [1.06-1.16]	<0.01	123/264

*Logistic regression was used in the analyses calculating the **OR of asthma at 13 years each time the sensitization score is increased by 1**
 § Total of children with complete data on sensitization and asthma

86 **Table S10:** Association between temporal sensitization patterns and asthma at 13 years of age
 87 adjusted for father’s asthma (all mothers have asthma), mother’s elevated sIgE, father’s elevated
 88 sIgE and mother’s smoking during 3rd trimester.
 89

Sensitization pattern	SPT			sIgE		
	aOR [95% CI]*	P-value	Nsensi/Ntotal	aOR [95% CI]*	P-value	Nsensi/Ntotal
Early transient	4.84 [0.77-30.25]	0.09	10/160	NA	NA	17/204
Late-onset	2.21 [0.53-9.13]	0.09	31/160	2.31 [0.59-9.00]	0.23	27/204
Persistent	8.61 [2.52-29.41]	<0.01	29/160	2.88 [0.98-8.45]	0.05	70/204

Complete data on SPT and asthma were available on 171 children; 22 had asthma.
 Complete data on sIgE and asthma were available on 215 children; 26 had asthma
 *never sensitized children used as control group