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Title page

Title: Is there a march from early food sensitization to later childhood allergic airway disease? Results from two prospective birth cohort studies

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Abstract page

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Title: Is there a march from early food sensitization to later childhood allergic airway disease? Results from two prospective birth cohort studies

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Abstract:

Background: The march from early aeroallergen sensitization to subsequent respiratory allergy is well established, but it is unclear if early life food sensitization precedes and further increases risk of allergic airway disease.

Objective: To assess the association between food sensitization in the first 2 years of life and subsequent asthma and allergic rhinitis by age 10-12 years.

Methods: We used data from two independent cohorts: the high-risk MACS (n=620) and the population-based LISAplus (n= 3094). Food sensitization was assessed at 6,

12 and 24 months in MACS and 24 months in LISAplus. Multiple logistic regressions were used to estimate associations between sensitization to food only, aeroallergen only or both and allergic airway disease.

Results: When compared to non-sensitized children, sensitization to food only at 12 months in MACS and 24 months in LISAplus was associated with increased risk of current asthma (aOR=2.2; 95%CI 1.1, 4.6 in MACS and aOR=4.9; 2.4,10.1 in LISAplus). Similar results were seen for allergic rhinitis. Additionally, co-sensitization to food and aeroallergen in both cohorts at any tested point was a stronger predictor of asthma (at 24 months, aOR=8.3; 3.7, 18.8 in MACS and aOR=14.4; 5.0, 41.6 in LISAplus) and allergic rhinitis (at 24 months, aOR=3.9;1.9,8.1 in MACS and aOR=7.6;3.0,19.6 in LISAplus).

Conclusions: In both cohorts, food sensitization (with or without aeroallergen sensitization) in the first two years of life increased the risk of subsequent asthma and allergic rhinitis. These findings support the role of early life food sensitization in the atopic march and suggest trials to prevent early onset have the potential to reduce the development of allergic airways disease.

Key words: Allergic rhinitis, Asthma, Atopy, Food Sensitization

Abbreviation:

aOR: Adjusted odd ratio

LISAplus: Influence of Life-style related factors on the development of the Immune System and Allergies in East and West Germany study

MACS: Melbourne Atopic Cohort Study

S-IgE: serum specific immunoglobulin E

SPT: Skin Prick Testing

Introduction

Over the past 50 years there has been a global epidemic of asthma, eczema and allergic rhinitis, especially in developed countries (1). Over the last 20 years, evidence suggests a second wave of the allergy epidemic with an increase in the prevalence of food allergies (2, 3). These allergic disorders pose a substantial health burden on affected individuals, their families and healthcare resources (4-6). Given the overlap between allergic disorders, the link between them has been a major research focus.

The "atopic march" refers to progression of allergic phenotype from early life eczema into later asthma and allergic rhinitis, which has been supported by many longitudinal and cross-sectional studies (7-9). There is increasing interest in these longitudinal relationships because the information could contribute in identifying early interventions and reduce burden of these disorders. Eczema is commonly associated with atopic sensitization, as assessed by either skin prick test (SPT) or serum specific IgE in vitro (s-IgE) (10). Previous studies suggest that eczema along with atopy is considered as a major risk factor for progression in the atopic march (11). Data from Melbourne Atopic Cohort Study (MACS) showed that children with atopic eczema in the first two years of life had a greater risk of asthma and allergic rhinitis at 6 and 7 years when compared with children with non-atopic eczema (9).

Although several epidemiological studies have shown that early aeroallergen sensitization is related to increased risk of allergic diseases in children (12-15) and adults (16), the role of food sensitization is less clear. Considering that food sensitization tends to develop earlier than aeroallergen sensitization, measuring food sensitization in the early years of life may allow earlier prediction of childhood and adolescent-onset allergic airway disease and potentially target intervention strategies in early life.

Food sensitization has been hypothesised to be related to development of other allergic diseases including asthma, allergic rhinitis (8, 17, 18) and food allergy (19). Although a number of epidemiological studies have assessed the association between food sensitization and subsequent asthma and/or allergic rhinitis up to seven years (12, 20, 21), only a few cohorts have assessed these associations beyond this age (22-24). However, concomitant early life eczema and/or wheeze have not been considered in most studies.

We conducted prospective analyses of two independent cohorts: the high-risk Australian based MACS cohort and the population based Influence of Life-style related factors on the development of the Immune System and Allergies in East and West Germany plus the influence of traffic emissions and genetics (LISAplus) cohort. We investigated the association between food sensitization, with or without aeroallergen sensitization, at different time points in the first two years of life and risk of allergic airway disease by age 10-12 years, whilst taking into account various confounding factors.

Methods

Study populations

MACS began as a randomized controlled trial investigating the effect of three different infant formulas (cow's milk, partially hydrolysed whey and standard soy formulas) introduced at the time of weaning on the occurrence of allergic diseases. A total of 620 infants born between 1990 and 1994 were recruited from antenatal clinics at the Mercy Maternity Hospital in Melbourne, Australia. Children were eligible if they had a first degree relative with asthma, eczema, hay fever or food allergy. Baseline information was collected during pregnancy.

Telephone-based interviews were conducted every 4 weeks until 15 months, at 18 months, 2 years then annually up to the age of 7 years, then at 12 and 18 years. At the 12 year follow-up, the mean (±SD) age of participants was 11.5±2 years. The study was approved by the Human Research Ethics committees of the Mercy Maternity Hospital and University of Melbourne. Written informed consent was obtained from all mothers and/or participants.

The baseline demographics of MACS participants have been published previously (9). Briefly, parents of MACS children were mainly Australian born (83% of fathers and 87% of mothers) and well educated (61% of fathers and 59% of mother had higher education).

Using data from randomized controlled trials to test additional hypotheses about the association between non-randomized exposures and outcomes determined during long-term follow-up is a well-established method. It is based on the testable assumption that the randomized intervention does not influence the associations of interest (25). A previous MACS publication showed that the randomization status (infant formula allocation) was not associated with the outcome of interest (26), therefore MACS continues as an observational study. Despite this, the effect of an intervention formula (by intention to treat at baseline) was considered as a potential confounder in all analyses.

LISAplus is a German population based birth cohort study that recruited 3094 neonates between 1997 and 1999 from the cities of Munich, Leipzig, Wesel and Bad Honnef. Questionnaires were completed by parents at birth, 6 months, 1, 1.5, 2, 4, 6 and 10 years of age. Details of the study design have been described elsewhere (27). The study was approval by the local Ethics Committees (Bavarian Board of

Physicians, University of Leipzig, and Board of Physicians of North-Rhine-Westphalia) and written parental consent was obtained.

The demographic characteristics for the initial LISAplus cohort have been described in previous publications (27).

Sensitization assessment

In MACS, Skin Prick Tests (SPT) were performed at 6, 12 and 24 months, according to a standard technique (28). Allergens tested included egg white, cow's milk, peanut, house dust mite *(Dermatophagoides pteronyssinus)*, rye grass (*Lolium perenne*) and cat dander (Bayer, Spokane, WA, USA). A positive histamine control (1 mg/mL) was used. SPTs were read at 15-20 minutes. Wheal size was measured by calculating the mean length of the longest and perpendicular wheal diameters (15). Sensitization was defined as wheal size of ≥2mm (29).

In LISAplus, blood samples were collected at the age of 2 years. Serum-specific IgE antibodies (s-IgE) were measured using a mix of common food allergens (FX5: hen's egg, cow's milk, peanut, wheat flour, soybean, and codfish). If the specific IgE exceeded 0.35 kU_A/L, egg white, milk protein and peanuts protein were tested separately. The inhalant allergens HX2 (mite), E1 (cat), MX1 (mold), RX1 (pollen) were tested separately. Standardised methods with the CAP System FEIA (Pharmacia Diagnostics, Freiburg, Germany) were used. Sensitization was defined as an IgE antibody level ≥0.35 kU_A/L.

Outcome definitions

Allergic outcomes were defined by questionnaire responses at age 10 year follow-up in LISAplus and at 12 year follow-up in MACS.

Current asthma

MACS defined current asthma as one or more episodes of asthma and/or the use of any asthma medications in the last 12 months. LISAplus defined current asthma as doctor diagnosis of asthma at the age of 10 years or intake of asthma medication during the past 12 months.

Current Allergic rhinitis

MACS defined current allergic rhinitis as one or more episodes of allergic rhinitis and/or the use of any treatment for allergic rhinitis in the last 12 months. LISAplus

defined allergic rhinitis as doctor diagnosis of seasonal and/or perennial rhinitis at the age of 10 years.

Confounder definitions

Eczema by the age at which the sensitization was assessed:

Defined as doctor diagnosis or treatment of rash with topical steroid (excluding nappy or scalp areas) by the age of SPT in MACS and as doctor diagnosis of eczema in the past 6 months and/or rash in the past 6 months asked at the age of 2 years in LISAplus.

Wheeze by the age at which the sensitization was assessed:

Defined if the response to the following question was >5 days "How many days of cough and/or chest rattle and/or wheeze has your child had in the past 4 weeks?" in MACS and according to the response to the following question "In the past 6 months, has your child had whistling or wheezy sound of breathing in the chest?" asked during the follow-up at age 2 years in LISAplus.

Statistical Analysis

Logistic regression models were constructed with asthma or allergic rhinitis as dependent variables and food and/or aeroallergen sensitization at 6, 12 or 24 months (in MACS) or at 24 months (in LISAplus) as independent variables. The predictive evaluation of sensitization was based on four groups: (1) no sensitization, (2) food sensitization only, (3) aeroallergen sensitization only and (4) sensitization to both food and aeroallergen. In MACS, the associations were evaluated at each time point separately, irrespective of previous status of sensitization. All models were adjusted for sex, maternal smoking during pregnancy, parental level of education, exclusive breastfeeding for at least 4 months (30) and eczema by the age of sensitization. The association between atopic sensitization and asthma was further adjusted for wheeze by the age at the assessment of sensitization.

Additional adjustment for formula allocation was performed in MACS analyses and for study center and family history of allergy (whether mother, father or a sibling ever had asthma, eczema or hay fever; asked at birth) in LISAplus. An interaction between allergic sensitization and family history of allergy was tested in LISAplus.

Results are presented as crude and adjusted Odd Ratios (OR) and 95% confidence intervals. All statistical tests were two sided with a p value of <0.05 considered as

statistically significant. STATA 13 (StataCorp, College Station TX) was used in all analyses in MACS and R version 3.1.0 was used for all analyses in LISAplus (31).

Results

Characteristics of the study populations

The characteristics for analyzed MACS participants are presented in **Table 1**. At all time points tested in MACS, egg white was the commonest food allergen (13%, 18% and 15% at 6, 12 and 24 months, respectively), while house dust mite was the most prevalent aeroallergen (5%, 12% and 23% at 6, 12 and 24 months, respectively. With the exception of maternal education and paternal smoking, MACS participants who did not attend the 12 year follow-up were similar on all demographic characteristics and early life sensitization compared to those who did attend **(see Table E1 in the Online Repository)**.

The characteristics for participants analyzed from LISAplus (630, 292, 138 and 120 participants from Munich, Leipzig, Bad Honnef and Wesel, respectively) are presented in **Table 1.** At 24 months in LISAplus, the major food allergens were egg white and milk protein (5% each) and the major aeroallergen was house dust mite (3%). Apart from parental education, maternal smoking, number of older siblings, a sibling ever having asthma and parental history of food allergy or hay fever, LISAplus participants who did not attend the 10 year follow-up were similar on other demographic characteristics and sensitization at 24 months compared to those who did attend **(see Table E1 in the Online Repository).**

Atopic sensitization and allergic airway diseases

Food sensitization and asthma and allergic rhinitis

In MACS, infants who were sensitized to food allergens without concurrent aeroallergen sensitization at 12 months had an increased risk of current asthma and allergic rhinitis compared to non-sensitized infants. While there were similar trends at 6 and 24 months, these were not significant (**Tables 2&3)**. Additionally, children who had co-sensitization to both food and aeroallergen at 6, 12 or 24 months had increased risk of current asthma and allergic rhinitis. These associations, at all three time points, appeared stronger than sensitization to food without sensitization to aeroallergen (**Tables 2&3)**.

In LISAplus, children who were sensitized to food without concurrent aeroallergen at 24 months had an increased risk of current asthma and allergic rhinitis compared to non-sensitized children (**Tables 2&3)**. Similar to MACS, the strongest effect on asthma and allergic rhinitis risk was observed in children who had co-sensitization to food and aeroallergen at 24 months (**Tables 2&3)**.

Aeroallergen only sensitization and asthma and allergic rhinitis

In MACS, sensitization to aeroallergen without food at 12 months, but not at 6 months was associated with increased risk of current asthma and allergic rhinitis (**Tables 2&3)**. Moreover, children who had aeroallergen without food sensitization at 24 months in both cohorts had increased risks of current asthma and allergic rhinitis and these associations were weaker than sensitization to both food and aeroallergen (**Tables 2&3)**.

None of the above associations were modified by family history of allergy in LISAplus study (i.e. *p* value of interaction was >0.1).

Discussion

Our study has shown that food sensitization in the first two years, independent of early life eczema and wheeze, predicts asthma and allergic rhinitis in later childhood. Additionally, co-sensitization to both food and aeroallergen was the strongest predictor at any time point tested. Our findings were mostly consistent across two cohorts, where data were available, with different populations in relation to co-sensitization to food and aeroallergen and sensitization to aeroallergen only. The MACS is an Australian cohort of children with a family history of allergic diseases while LISAplus is a German population-based cohort. Interestingly, our findings were similar among those with and without a family history within the LISAplus study. Earlier studies have established the role of aeroallergen sensitization on development of allergic airway diseases (13-16). We established that food sensitization itself was related to subsequent increased risk of asthma and allergic rhinitis. The current analysis compared the effect of different mutually exclusive groups of atopic sensitization on asthma and allergic rhinitis in later childhood allowing us to draw stronger conclusions on the different patterns of sensitization. Few studies have investigated the role of early life food sensitization on development of asthma and

allergic rhinitis beyond the age of 7 years (22-24). Bekkers and colleagues (24) showed that egg, but not cow's milk sensitization at 12 months was associated with increased risk of asthma up to the age of 10 years. However, whether the observed effect was due to sensitization to food alone without concurrent aeroallergen was not assessed. Additionally, potential confounding by early life eczema and/or wheeze was not considered in this analysis.

Our study showed that food without aeroallergen sensitization at 24 months was associated with increased risks of asthma and allergic rhinitis in the LISAplus study but not in MACS. This appears to be due, at least in part, to only a small number of MACS participants only having food sensitization at 24 months compared to the LISAplus study. In contrast, findings related to aeroallergen without food sensitization and co-sensitization to foods and aeroallergen were consistent across both cohorts.

We found also that children co-sensitized to common foods and aeroallergen at any time point tested had a markedly higher risk of developing respiratory allergic diseases than sensitization to food or aeroallergen alone when compared to non-sensitized children. Few longitudinal studies have assessed the association between co-sensitization to food and aeroallergen and development of atopic diseases in childhood. A German study by Illi *et al.* (32) showed that concurrent sensitization to food and aeroallergen was the strongest predictor for asthma up to school age. In an Australian study, Garden *et al.* (23) found that mixed food and inhalant sensitization phenotype had the strongest associations with allergic disease at the age of 8 years, and particularly with asthma. However, co-manifestation of early life wheeze and/or eczema was not been taken into account in the analysis and there was a shorter period of follow-up.

Eczema in early life is commonly associated with high levels of food specific IgE (33), and has been associated with increased risk of later asthma and allergic rhinitis (9). Similarly, wheeze has been related to food specific IgE (34). When early life eczema and/or wheezing, reported at the same age of testing for atopic sensitization, were considered in our analysis, the associations between atopic sensitization and allergic outcomes did not change significantly. This suggests that the role of early life sensitization on later childhood allergic airway diseases is independent of eczema and/or wheezing.

The strengths of this study are that we have analyzed longitudinal data from two independent cohorts with long periods of follow-up (extending from infancy to later childhood), the relatively large sample sizes and early objective assessment of sensitization to common allergens. These cohorts were from two different regions of the world, but both were high income countries with high prevalences of food sensitization (35) and allergic diseases (36-38). It is often assumed that results from a high-risk cohort may not be applicable to the general population, but interestingly our results were similar across the two cohorts. Also, family history of allergic diseases did not modify our associations in the population-based cohort suggesting that family history may not be a major modifier of risk and that other factors should be considered, for example environmental exposures.

This study has a number of limitations. Our analyses were based on longitudinal data and loss to follow-up needs to be considered when interpreting the results as it could be a potential source of bias. However, these studies achieved a 57% attendance at the 10 year follow-up in LISAplus and 59% attendance at the 12 year follow-up in MACS. In addition, apart from parental education and paternal smoking, there were no significant demographic and/or early sensitization differences between children who did and did not attend in either cohort. Although the reported paternal history of food allergy and hay fever was higher in those who attended the 10 years follow-up in LISAplus, this was unlikely to bias findings. Another possible limitation is that the definitions of asthma and allergic rhinitis were based on questionnaire data. However, these definitions have been commonly used in epidemiological studies (20, 39). The findings of this study could have been strengthened if participants were examined for objective evidence of asthma and allergic rhinitis. Moreover, we were unable to establish the associations between specific allergen sensitization and subsequent development of asthma and allergic rhinitis due to limited statistical power.

Previous studies have observed that positive SPT reactions are likely to be smaller in infants and children younger than two years (40) presumably because of a relative lack of allergen-specific IgE and skin reactivity (41). Therefore, in the MACS cohort, a 2 mm cut-off point was used to define positive skin prick test reactions at 6, 12 and 24 months (42).

We acknowledge that food sensitization was assessed by SPT in MACS and by serum IgE in LISAplus. These two methods are commonly used to evaluate sensitization in epidemiological studies (43). Many studies have assessed sensitization as a predictor

of allergic diseases, either by SPT (12, 22, 23) or s-IgE (12, 20, 24, 44). A recent study by Ro *et al.* (45) showed that the predictive value of SPT and s-IgE performed at 2 years of age was generally comparable in predicting allergic diseases in later childhood.

In conclusion, assessment of food sensitization in infants provides valuable information on the risk of later childhood asthma and allergic rhinitis. Additionally, we provide evidence for the role of early life food sensitization with or without co-sensitization to aeroallergen, independent of early life eczema, on the atopic march. Developing interventions that prevent early life food sensitization, such as food allergen avoidance or dietary modification, may reduce the likelihood of atopic march to asthma and allergic rhinitis occurring.

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Conflict of interest

No conflict of interest to declare.

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Table 1: Characteristics of participants analyzed from the MACS and LISAplus studies

	MACS			LISAplus
	6 Months	**12 Months**	**24 Months**	**24 Months**
	N*=335	N*=343	N*=307	N= 1180
	n (%)	n (%)	n (%)	n (%)
Sex				
Male	180 (54)	180 (52)	163 (53)	617 (52)
Female	155 (46)	163 (48)	144 (47)	563 (48)
Family history of allergy				
Yes	(100)	(100)	(100)	734 (62)
Maternal smoking during pregnancy				
Yes	18 (5)	20 (6)	19 (6)	148 (13)
Parental education level				
high	247 (74)	246 (72)	227 (74)	875 (74)
Exclusive breastfeeding ≥ 4m				
No	187 (56)	192 (56)	170 (55)	459 (39)
Yes	147 (44)	151 (44)	137 (45)	721 (61)
Current allergic disease**				
Asthma				
No	255 (76)	261 (76)	235 (76)	1118 (95)
Yes	80 (24)	82 (24)	72 (24)	62 (5)
Allergic rhinitis				
No	212 (63)	214 (62)	193 (63)	1057 (90)
Yes	123 (37)	129 (38)	114 (37)	123 (10)

* N represents the number of participants who had available data on both sensitization and allergic diseases (asthma and allergic rhinitis).

**At 10Y in LISAplus and 12Y in MACS.

Table 2: The association between food only, aeroallergen only and both food and aeroallergen sensitization and asthma in MACS and LISAplus.

Cohort	Atopic sensitization	n (%)	Asthma‡				
			Prevalence* (%)	Crude OR (95%CI)	*p*	Adjusted** OR (95% CI)	*p*
MACS	**6 months**						
	Non-sensitized	251 (75)	18	-		-	
	Food only	49 (15)	31	1.9 (1.0, 3.9)	0.05	1.8 (0.9, 3.8)	0.08
	Aero only	14 (4)	36	2.5 (0.8, 7.7)	0.11	2.2 (0.7, 7.4)	0.18
	Food and aero	21 (6)	67	8.9 (3.4, 23.3)	**<0.01**	6.1 (2.3, 16.7)	**<0.01**
	12months						
	Non-sensitized	233 (68)	15	-		-	
	Food only	48 (14)	31	2.6 (1.3, 5.2)	**<0.01**	2.2 (1.1, 4.6)	**0.03**
	Aero only	25 (7)	48	5.2 (2.2, 12.4)	**<0.01**	5.1 (2.1, 12.3)	**<0.01**
	Food and aero	36 (11)	53	6.3 (2.9, 13.3)	**<0.01**	5.6 (2.5,12.3)	**<0.01**
	24 months						
	Non-sensitized	192 (63)	13	-		-	
	Food only	22 (7)	23	2.1 (0.7, 6.1)	0.19	1.7 (0.6, 5.4)	0.34
	Aero only	53 (17)	40	4.6 (2.3, 9.2)	**<0.01**	4.9 (2.4, 10.2)	**<0.01**
	Food and aero	39 (13)	54	8.2 (3.8, 17.4)	**<0.01**	8.3 (3.7,18.8)	**<0.01**
LISAplus	**24 months**						
	Non-sensitized	1041 (88)	3	-		-	
	Food only	94 (8)	14	4.6 (2.3,9.1)	**<0.01**	4.9 (2.4,10.1)	**<0.01**

Aero only	25 (2)	24	9.1 (3.4,24.1)	**<0.01**	10.2 (3.6,28.5)	**<0.01**
Food and aero	20 (2)	40	19.2 (7.4,49.8)	**<0.01**	14.4 (5,41.6)	**<0.01**

‡At age 10 year follow-up in the LISAplus study and at 12 year follow-up in the MACS study.

*Prevalence refers to the proportion of individuals who developed asthma in each sensitization group.

** Adjusted for maternal smoking during pregnancy, parental level of education, sex, exclusive breast feeding for at least 4 months, eczema and wheeze by the age of sensitization assessment and formula allocation (in MACS only) and study center and family history of allergy (in LISAplus only).

Table 3: The association between food only, aeroallergen only and both food and aeroallergen sensitization and allergic rhinitis in MACS and LISAplus

Cohort	**Atopic sensitization**	n (%)	**Allergic Rhinitis‡**				
			Prevalence* (%)	Crude OR (95%CI)	*p*	Adjusted OR (95% CI)	*p*
MACS	**6 months**						
	Non-sensitized	251 (75)	34	-		-	
	Food only	49 (15)	38	1.2 (0.6, 2.2)	0.61	1.1 (0.5, 1.9)	0.93
	Aero only	14 (4)	43	1.4 (0.5, 4.2)	0.51	1.3 (0.4, 4.0)	0.67
	Food and aero	21 (6)	62	3.1 (1.2, 7.8)	**0.01**	2.8 (1.0, 7.7)	**0.04**
	12months						
	Non-sensitized	233 (68)	30	-		-	
	Food only	48 (14)	48	2.1 (1.1, 3.9)	**0.01**	2.1 (1.1, 3.9)	**0.02**
	Aero only	25 (7)	52	2.5 (1.1, 5.8)	**0.02**	2.4 (1.0, 5.6)	**0.03**
	Food and aero	36 (11)	62	3.8 (1.8, 7.8)	**< 0.01**	3.6 (1.7, 7.7)	**< 0.01**
	24 months						
	Non-sensitized	192 (63)	30	-		-	
	Food only	22 (7)	36	1.4 (0.5, 3.4)	0.52	1.3 (0.5, 3.3)	0.60

	Aero only	53 (17)	45	1.9 (1.1, 3.6)	**0.03**	1.9 (0.9, 3.5)	**0.05**
	Food and aero	39 (13)	63	3.9 (1.9, 8.0)	< **0.01**	3.9 (1.9, 8.1)	< **0.01**
LISAplus	**24 months**						
	Non-sensitized	1041 (88)	8	-		-	
	Food only	94 (8)	20	2.7 (1.6,4.7)	< **0.01**	2.8 (1.6,4.8)	< **0.01**
	Aero only	25 (2)	24	3.4 (1.3,8.8)	**0.01**	3.0 (1.1, 7.9)	**0.03**
	Food and aero	20 (2)	50	10.8 (4.4, 26.7)	< **0.01**	7.6 (3.0, 19.6)	< **0.01**

‡At age 10 year follow-up in the LISAplus study and at 12 year follow-up in the MACS study.

*Prevalence refers to the proportion of individuals who developed allergic rhinitis in each sensitization group.

** Adjusted for maternal smoking during pregnancy, parental level of education, sex, exclusive breast feeding for at least 4 months, eczema by the age of sensitization assessment and formula allocation (in MACS only) and study center and family history of allergy (in LISAplus only)