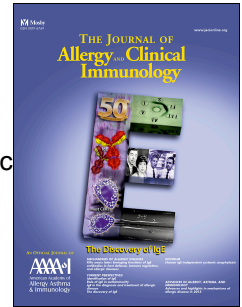


Accepted Manuscript

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PII: S0091-6749(17)30221-X

DOI: [10.1016/j.jaci.2016.12.973](https://doi.org/10.1016/j.jaci.2016.12.973)

Reference: YMAI 12630

To appear in: *Journal of Allergy and Clinical Immunology*

Received Date: 21 June 2016

Revised Date: 12 December 2016

Accepted Date: 19 December 2016

Please cite this article as: Asarnoj A, Hamsten C, Lupinek C, Melén E, Andersson N, Anto JM, Bousquet J, Valenta R, van Hage M, Wickman M, Prediction of peanut allergy in adolescence by early childhood storage protein-specific IgE signatures: the BAMSE population-based birth cohort, *Journal of Allergy and Clinical Immunology* (2017), doi: 10.1016/j.jaci.2016.12.973.

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Prediction of peanut allergy in adolescence by early childhood storage protein-specific IgE signatures: the BAMSE population-based birth cohort

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Short title: Evolution of peanut allergy during childhood

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Key words

Peanut allergy; allergen; Ara h 2; component resolved diagnostics; IgE; molecular allergology; sensitization; birth cohort; BAMSE

Funding

Supported by the Swedish Asthma and Allergy Research Foundation; Stockholm County Council; the Swedish Research Council of Health, Working Life and Welfare; the Swedish Research Council, the Swedish Society of Medicine, the Swedish Heart-Lung Foundation; the Swedish Cancer and Allergy Foundation; the Konsul Th Berg's Foundation, the Magnus Bergvall Foundation, the Swedish Association for Allergology, the European Commission's Seventh Framework Program MeDALL under grant agreement no. 261357, and in part by grant F4605 of the Austrian Science Fund (FWF).

51 **Author Contributions**

52 Data acquisition: AA, CL, EM, NA, RV, MvH, MW;

53 Technical support: CH, CL, NA, RV;

54 Material support: CL, RV, MvH;

55 Manuscript writing: AA, CH, MvH, MW;

56 Critical revision of the manuscript: EM, JA, JB, RV;

57 Study supervision: EM, MvH, MW

Abbreviations

Ara h: *Arachis hypogaea*

PR-10: Pathogenesis-related protein family 10

LTP: Lipid transfer protein

95% CI: 95% Confidence interval

OAS: Oral allergy syndrome

DBPCFC: Double-blind placebo-controlled food challenge

66

67 **Capsule summary (34 words)**

68 IgE signatures to peanut allergen measured in early childhood allow predicting the likelihood of peanut
69 allergy in adolescence. Peanut symptoms and Ara h 2 IgE >2.0 ISU-E at 4 years predict peanut allergy in
70 adolescence.

To the Editor:

One of the most frequent and severe forms of food allergy is caused by peanuts¹. IgE reactivity to peanut storage proteins, in particular to Ara h 2, is associated with systemic reactions². However, in some regions the lipid transfer protein Ara h 9 is an important allergen molecule whereas in birch endemic areas, the PR-10 protein Ara h 8 is a more common cross-reactive component³.

In the Isle of Wight birth cohort, peanut allergy showed an early onset⁴. Interestingly, the percentage of symptomatic patients did not increase but an increase in peanut extract sensitization was noted until adolescence⁴. The development of IgE antibody reactivity to the different peanut allergen molecules as well as how IgE sensitization to these molecules contributes to development of peanut symptoms has not yet been explored.

In this study, the evolution of sensitization to peanut allergen molecules from early childhood to adolescence was investigated for the first time.

We used a random subset of 778 children from the Swedish BAMSE birth cohort (N=4089)⁵ where complete relevant questionnaire data from baseline (2 months), 1, 2, 4, 8 and 16 years and blood samples from 4, 8 and 16 years were available. For some of the analyses, this *population-based study group* was enriched with additional 84 peanut extract-sensitized children (at 4, 8 and/or 16 years) from the same cohort. Thus, our *peanut-enriched study group* included 862 children (778 from the population-based study group enriched with 84 peanut sensitized children) (Figure E1). Serum samples were analyzed for IgE antibodies to peanut extract, and at 8 years of age also to Ara h 2, by ImmunoCAP (Thermo Fisher AB, Uppsala, Sweden) and for peanut allergen molecules (Ara h 1, h 2, h 3, h 6, h 8 and h 9) using a modified allergen chip based on ISAC technology (Thermo Fisher)⁵. The correlation factor (ρ) between the IgE levels to Ara h 2 at 8 years measured with microarray and ImmunoCAP was high: 0.92. A linear regression with ImmunoCAP and microarray Ara h 2 IgE levels was performed to calculate corresponding values. Detailed information on methods and statistics is provided in the Online Repository at www.jacionline.org.

In the *population-based study group*, the frequencies of sensitization to storage proteins showed little change from 4 to 16 years, but sensitization to the birch pollen homologous allergen molecule Ara h 8 increased at each time point (Figures 1A and 1B). Any reported systemic symptom to peanut in combination with peanut storage protein sensitization increased from 1.4% at 8 years to 3.0% at 16

years ($p=0.03$, Table E1). The prevalence rates and trajectories, i.e. onset, persistence and transition of IgE reactivity, as well as IgE-levels to peanut extract and the different peanut allergen molecules are displayed in Figures 1A and 1B.

Due to the substantial overlap of sensitization to the peanut storage proteins (Figures E2 and E3), no multivariate logistic regression estimates for IgE reactivity to each storage protein in relation to allergic symptoms to peanut could be performed. Of the 54 Ara h 2-sensitized children at 16 years in the *peanut-enriched study group*, 45 (83%) were already sensitized at 4 years and 49 (91%) reported symptoms to peanut at 16 years. Only one participant had *de novo* Ara h 2 sensitization (0.8 ISU-E) after 8 years (data not shown). The high rate of storage protein sensitization at 4 years among children developing peanut allergy during the first 16 years of life raises the important question of prevention.

In children with both peanut and birch pollen sensitization, one fraction was mono-sensitized to Ara h 8 (Figure 2). These children reported no peanut symptoms or only oral allergy syndrome (OAS) at 16 years of age. We have previously reported that sensitization to Ara h 8 is associated with mild OAS or peanut tolerance⁶. Interestingly in this study, most children with onset of peanut sensitization after 4 years had IgE reactivity to Ara h 8 only and not to the storage peanut proteins, and were mostly asymptomatic or without report of any systemic symptoms^{4,6}. Another fraction of children was sensitized to Ara h 2 (regardless co-sensitization to Ara h 8). At 16 years, peanut symptoms at exposure were reported in 91% of these Ara h 2 sensitized participants.

The predicted longitudinal likelihood of reporting peanut allergy at 16 years in the *peanut-enriched study group* ($n=862$) was plotted in relation to IgE-levels to Ara h 2 (Figure E4A, incident symptoms Figure E4B), peanut extract (Figure E4C) as well as to the number of sensitizing peanut allergen molecules 4 years of age (Figure E4D). An IgE-level of >2.0 ISU-E to Ara h 2, >15.5 kU_A/L to peanut extract or IgE reactivity (≥ 0.3 ISU-E) to at least 4 peanut allergen molecules at 4 years of age corresponded to a 95% likelihood of reporting peanut symptoms 12 years later.

Due to the large number of children investigated in the BAMSE cohort it was not possible to verify peanut symptoms with the double-blind placebo-controlled food challenge (DBPCFC). However, the calculated longitudinal peanut extract-specific IgE level at 4 years (15.5 kU_A/L) with a 95% likelihood of peanut symptoms at 16 years is very similar to the one reported in a cross-sectional clinical study by Sampson and colleagues (15 kU_A/L) where DBPCFC were included⁷. Several previous DBPCFC studies

demonstrate that even low Ara h 2 IgE-levels are associated with peanut allergy in almost all cases, which was shown in our study as well although most other studies measured Ara h 2 IgE with the ImmunoCAP method^{2, 7, 8}. As the results in ISAC are semiquantitative and may be influenced by blocking IgG antibodies, they cannot simply be transferred to ImmunoCAP. We made a comparison with ImmunoCAP values at 8 years of age and in the BAMSE cohort, an Ara h 2 level of 2.0 ISU-E in microarray corresponded to 4.1 kU/L in ImmunoCAP (data not shown).

In summary, our study is the first to examine the onset and persistence of IgE sensitization to peanut allergen molecules in relation to peanut symptoms from preschool age to adolescence. We identified two distinct phenotypes in the development of peanut allergy from childhood to adolescence. One phenotype develops early in life and is related to Ara h 2 sensitization and risk of systemic reactions after peanut exposure. Children with this phenotype rarely outgrow their symptoms. The second phenotype, which starts later in childhood, is related to Ara h 8 sensitization and non-systemic reactions after peanut ingestion. Furthermore, we show that in preschool children, Ara h 2 sensitization or polysensitization to peanut storage proteins are superior in predicting peanut allergy in adolescence compared to peanut extract sensitization. We suggest that measuring IgE to peanut allergen molecules may help clinicians improve the diagnosis and prognosis of peanut allergy.

Acknowledgements

We would like to thank all participating families and the staff in the BAMSE study as well as the staff at the laboratory of Professor Rudolf Valenta. Thermo Fisher Scientific kindly provided the ImmunoCAP singleplex reagents for the study.

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Figure legends

Figure 1. Evolution of sensitization (IgE $\geq$ 0.3 ISU-E or $\geq$ 0.35 kU/L, respectively) prevalence (**A**) and IgE-levels among sensitized individuals (**B**) to peanut allergen molecules and peanut extract at 4, 8 and 16 years of age in a population-based study group. N=778.

Footnote:

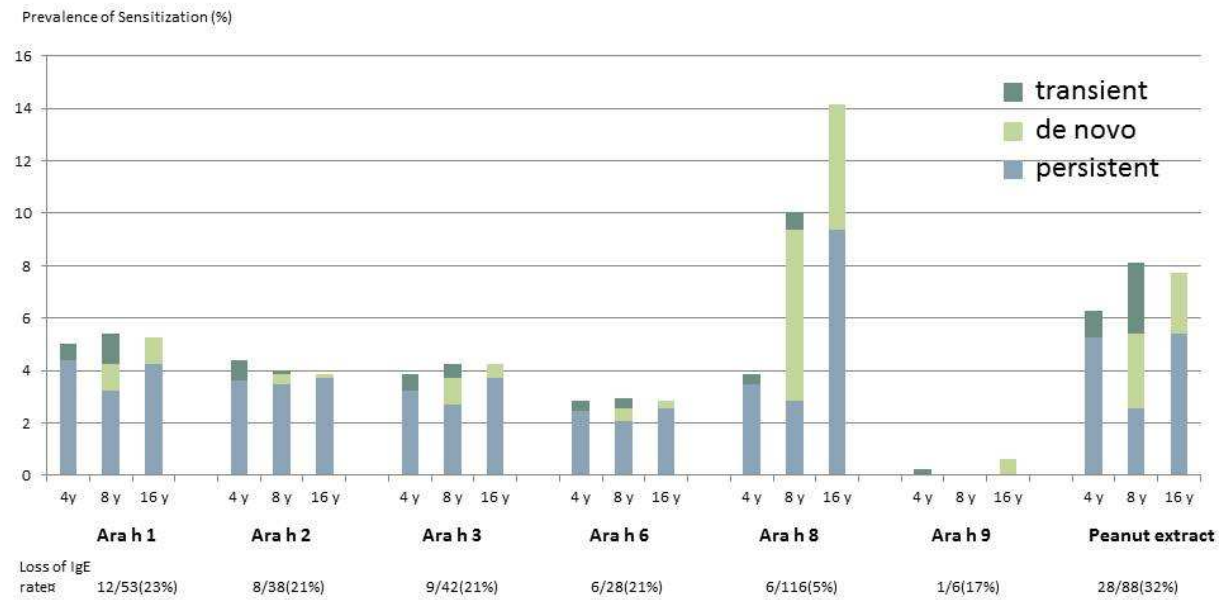
1A: $\propto$ proportion permanently desensitized if once sensitized to the specific allergen molecule (remission).

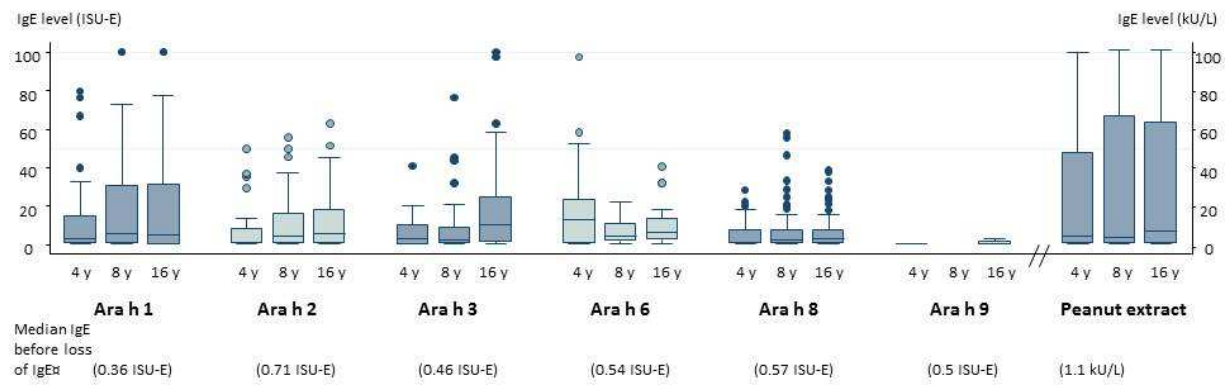
for comparison with Ara h 8 sensitization; Sensitization rates to birch Bet v 1 were 12.6%, 17.5% and 25.8% at 4, 8 and 16 years of age, respectively.

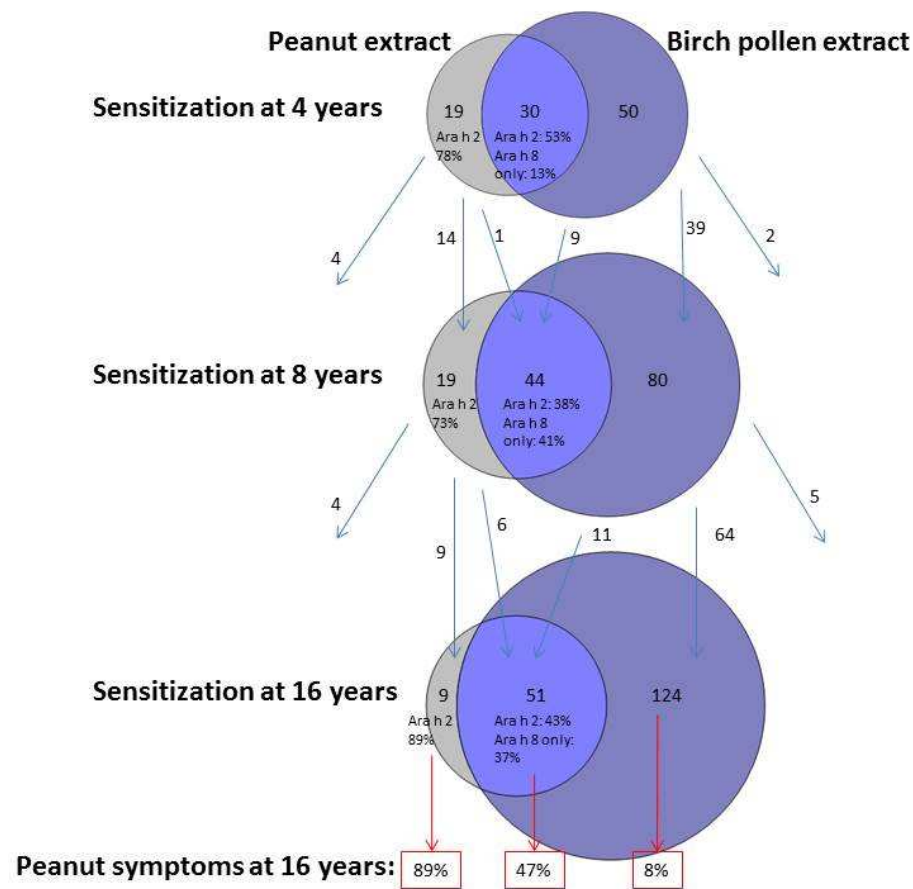
1B: *IgE levels to peanut allergen molecules and peanut extract did not differ significantly among sensitized individuals in the population-based study group (n=778) compared to the additional peanut extract sensitized participants (n=84).

Figure 2. Peanut and birch pollen extract sensitization overlap measured with ImmunoCAP (≥ 0.35 kU_A/L) at 4, 8 and 16 years of age. Proportion of Ara h 2 sensitization and Ara h 8 monosensitization in the subgroups measured with the MeDALL chip (≥ 0.3 ISU-E). N=778 (population-based study group)

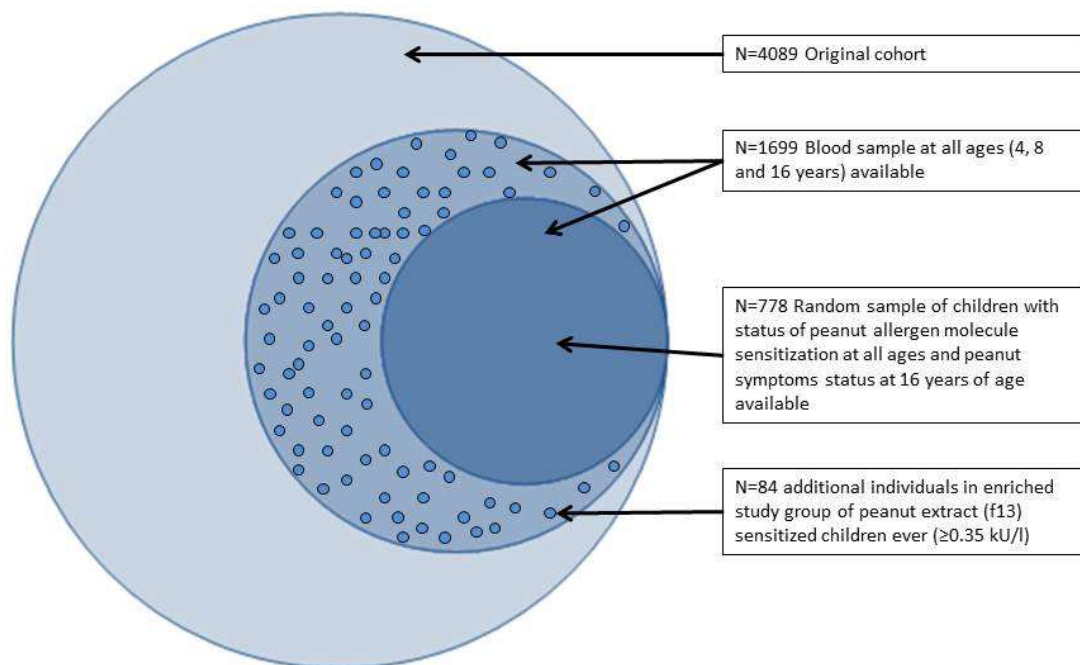
Footnote: *p-values from t-test on log transformed values, not including the 0-values in each symptom subgroup

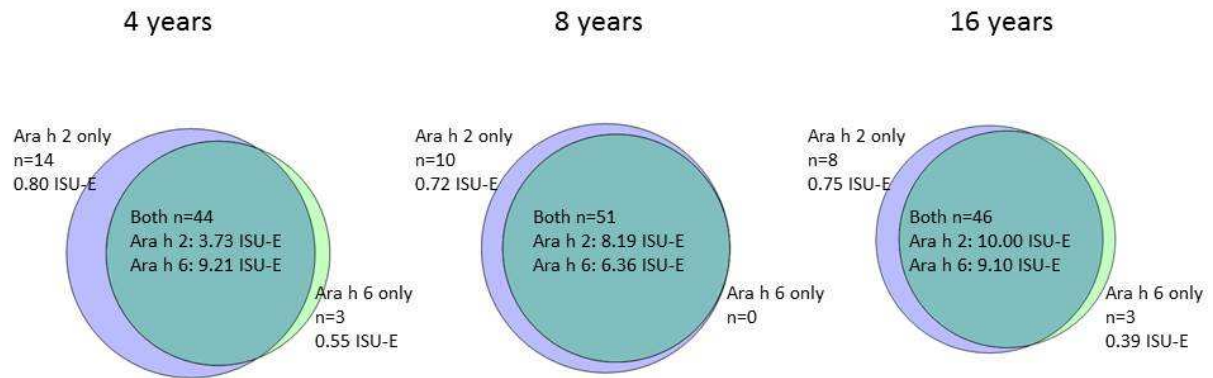




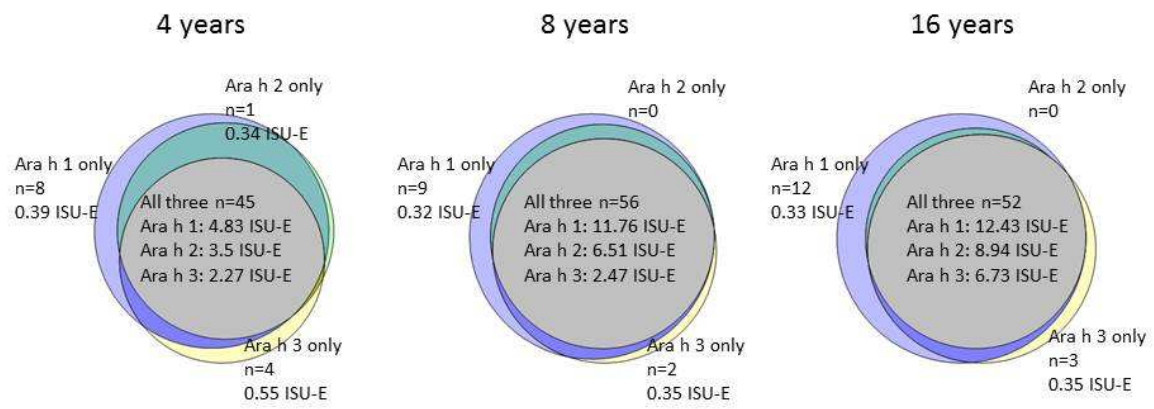


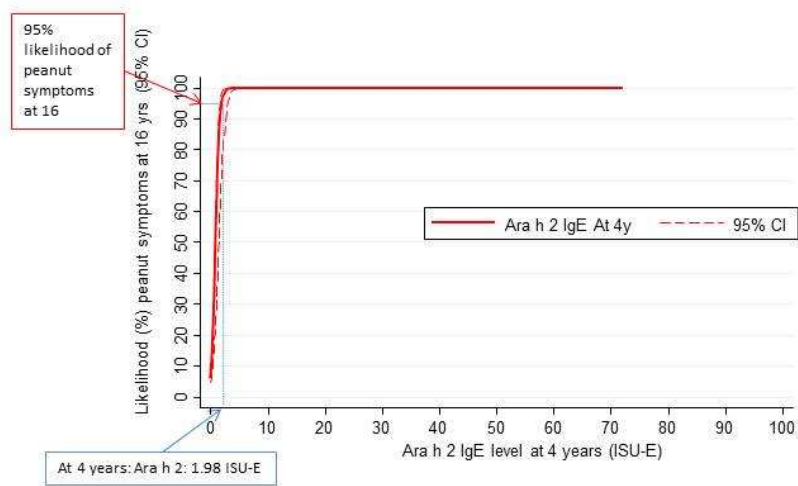
Study population

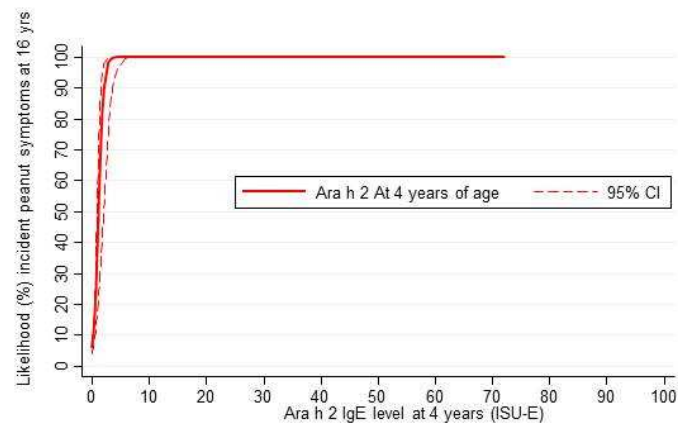


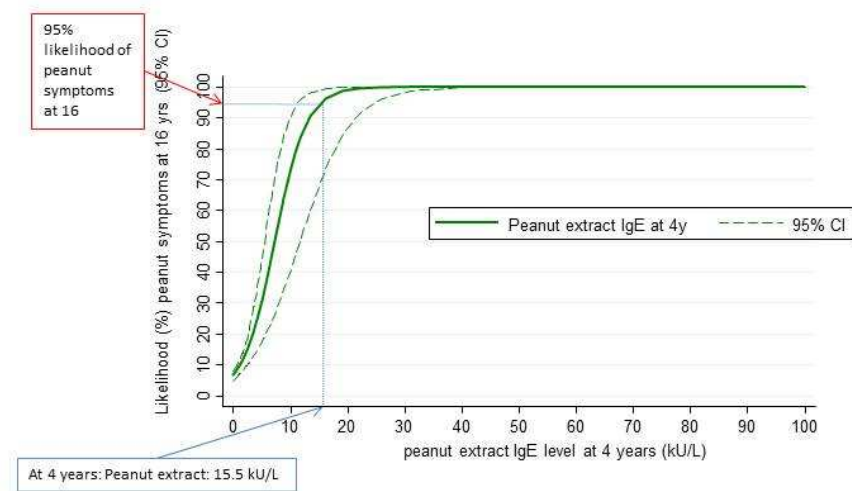


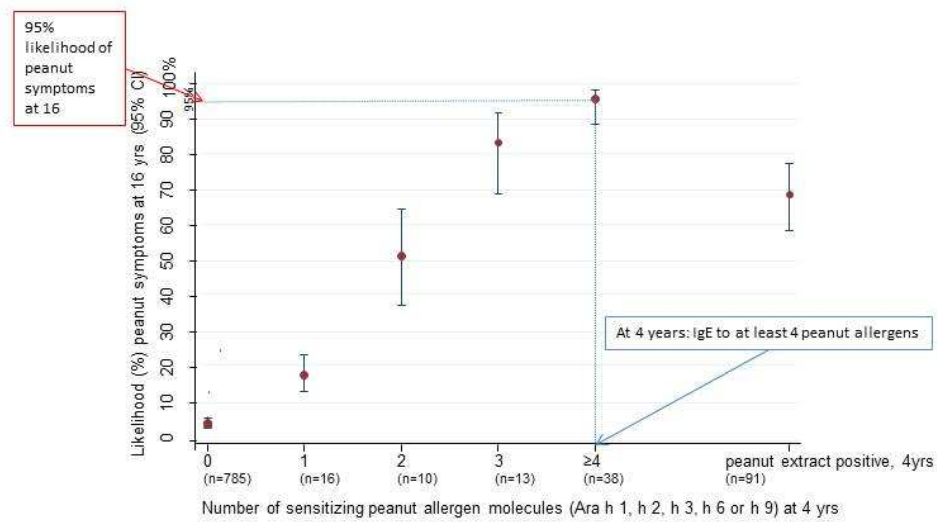
E3











Online Repository text

Methods

Study participants

BAMSE is an unselected population-based birth cohort study of 4089 children¹. For this study, a questionnaire was available from baseline (2 months), 1, 2, 4, 8 and 16 years. Blood was drawn at 4, 8 and 16 years. Sera were available for 64%, 60%, and 62% of the population.

In 1699 children, 42% of the original cohort, blood samples were available from all three clinical follow ups (4, 8 and 16 years). In this group of children, a random subset of 800 was collected of which 778 had complete data on peanut allergy at 16 years and sufficient serum volumes for analysis, denoted as the *population-based study group*.

For some of the analyses, this *population-based study group* was enriched with all remaining peanut extract-sensitized children (measured with ImmunoCAP (f13) at 4, 8 and/or 16 years of age) among the group of 1699. This resulted in 84 additional children for which complete peanut symptom data at 16 years of age and sufficient volume of serum for analysis were available. Thus, our *peanut-enriched study group* included 862 children (778 from the population-based study group enriched with 84 peanut sensitized children) (Figure E1).

Permission for the study was obtained from the Regional Ethical Review board at Karolinska Institutet at each follow up and parents of participating children gave their informed consent. At 16 years, the participating teens gave their separate consent.

Definition of symptoms

All data on peanut symptoms were collected through questionnaires answered by the children's parents at 1, 2, 4, 8 and 16 years. Specific organ symptoms to peanut were asked at 8 and 16 years.

Peanut allergy at 1, 2 and 4 years: Did your child ever have adverse reactions to food or drink, such as vomiting, diarrhea, eczema, nettle rash, itch or swelling of lips or eyelids, runny nose or asthma? ("Yes" and "peanut" indicated).

Peanut allergy at 8 years: Any of the following symptoms to peanut indicated: “Nose/Eye problems”, “Itching in mouth”, “Trouble breathing”, “Vomiting or diarrhea”, “Eczema”, “Nettle rash”, “Avoided foodstuff because of previous adverse reaction”.

Peanut allergy at 16 years: Any of the following symptoms to peanut indicated: “Difficulty breathing, asthma, cough”, “Itchy nose, stuffy nose, runny nose, itchy eyes”, “Nettle rash covering most of the body”, “Nettle rash that covered less”, “Vomiting, stomach pain”, “Swelling in face, eyelids, lips”, “Hoarseness, indistinct speech”, “Swollen feeling in mouth, throat”, “Itch in mouth, throat, ears”, “Pronounced fatigue, decreased awareness” and “Unconsciousness”.

Individuals with a positive answer to “Itch in mouth, throat, ears” and negative to all other symptoms were regarded as having local symptoms only (Oral Allergy Syndrome=OAS). Systemic peanut symptoms were defined as at least one positive answer to “Difficulty of breathing, asthma, cough” or “Nettle rash covering most of the body” or “Vomiting, stomach pain” or “Swelling in face, eyelids, lips” or “Hoarseness, indistinct speech” or “Pronounced fatigue, decreased awareness” or “Unconsciousness”.

Allergen-specific IgE measurement

Serum samples were analyzed with ImmunoCAP (Thermo Fisher AB, Uppsala, Sweden) for allergen-specific IgE antibodies to peanut extract (f13) and at 8 years of age also to Ara h 2. A positive test was defined as ≥ 0.35 kU_A/L. An IgE antibody level >100 kU_A/L was given the value of 101 kU_A/L in statistical evaluations and an IgE level <0.35 was set to 0.

IgE reactivity to the peanut allergen molecules (Ara h 1, Ara h 2, Ara h 3, Ara h 6, Ara h 8 and Ara h 9) was analyzed using a modified allergen chip based on ISAC technology (Thermo Fisher) developed in the MeDALL FP7-funded research programme². The cut-off was set at 0.3 ISU-E.

Biases

The diagnosis of peanut allergy was based on clinical symptoms and no food challenge was carried out, since the different peanut allergy phenotypes in the study population were not yet known at sampling and, for logistic reasons, it was not possible to perform food challenges in all 862 participants.

Sample size

No sample size was calculated as this is an exploratory study.

Statistical methods

Results are expressed as numbers and proportions (%). T-test of proportions was used for comparison of prevalence rates between groups. Group IgE levels are expressed as median value and range. T-test on log-transformed values was used for group comparisons of IgE levels. Odds Ratios (ORs) for symptoms to peanut at 16 years in relation to sensitization at 4 and 8 years were estimated using logistic regression models and 95% confidence intervals. Fitted predicted probability estimates were plotted according to the IgE-level (ISU-E and kU_A/L) to peanut allergen molecules or peanut extract (respectively) per participant, using the results from the logistic regression. The correlation factor (rho) between the IgE levels to Ara h 2 at 8 years measured with microarray and ImmunoCAP was high: 0.92. A linear regression with ImmunoCAP and microarray Ara h 2 IgE levels was performed to calculate corresponding values. 95% OR CI not including 1 and p-values <0.05 were considered significant. Analysis with logistic regression in order to investigate peanut symptoms in relation to sensitization to different peanut allergen molecules could not be performed due to substantial co-sensitization between different peanut storage proteins. All statistical analyses were performed with STATA Statistical Software (release 14.0; StataCorp, College Station, Texas, USA).

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Table E1. Prevalence of reported symptoms to peanut at 4, 8 and 16 years of age. Type of symptom specified at 8 and 16 years of age. N=778 (population based).

	1 year	2 years	4 years	8 years	16 years
Reported symptoms to peanut	3/769(0.4%)	6/777 (0.8%)	24/760 (3.2%)	48/773 (6.2%)	53/778 (6.8%)
Sensitization to peanut extract	n.a.	n.a.	49/778 (6.3%)	63/773 (8.2%)	61/778 (7.8%)
Peanut symptomatic and sensitized to peanut extract	n.a.	n.a.	17/760 (2.2%)	32/768 (4.2%)	32/778 (4.1%)
Sensitization to peanut storage protein	n.a.	n.a.	44/778 (5.7%)	42/778 (5.8%)	48/778 (6.2%)
Peanut symptomatic and sensitized to peanut storage proteins	n.a.	n.a.	17/760 (2.2%)	27/773 (3.5%)	30/778 (3.9%)
Peanut systemic symptom(s) and sensitized to peanut storage proteins	n.a.	n.a.	n.a.	11/773 (1.4%)	23/778 (3.0%)
				p=0.03	
Type of symptom*					
Upper resp	n.a.	n.a.	n.a.	9/28 (32.1%)	6/53 (11.3%)
Oral cavity	n.a.	n.a.	n.a.	14/28 (50.0%)	37/53 (69.8%)
Lower resp	n.a.	n.a.	n.a.	11/28 (39.3%)	20/53 (37.7%)
Gastrointestinal	n.a.	n.a.	n.a.	5/28 (17.9%)	9/53 (17.0%)
Urticaria	n.a.	n.a.	n.a.	8/28 (28.6%)	13/53 (24.5%)
Circulatory	n.a.	n.a.	n.a.	n.a.	1/53 (1.9%)
≥2 systemic symptoms	n.a.	n.a.	n.a.	4/28 (14.3%)	16/53 (30.2%)
				p=0.11	

n.a.= information not available

resp=respiratory

*only 28 of the 48 peanut symptomatic children specified type of symptom at 8 years of age.

Table E2. Baseline (at median age 2 months) and age one year characteristics of the population-based study group (N=778, number of missing values in each row: 0-7) and the peanut extract sensitized enriched study group (N=862, number of missing values: 0-12), compared to children in the original cohort (N=4089, number of missing values: 0-170).

Variables	Study base cohort (N=4089)		Study population, population based (N=778)			Additional peanut extract sensitized group (N=84)		
	n	%	n	%	p-value	n	%	p-value
Female	2024	49.5	391	50.3	0.70	41	48.8	0.90
Parental history of allergy	1200	29.7	256	33.0	0.064	34	40.5	<0.01
Breastfed ≥ 4 months	3116	79.5	609	79.3	0.95	68	82.9	0.45
Parental smoking	855	21.0	158	20.4	0.70	20	23.8	0.53
Young mother at birth (≤ 25 years)	319	7.8	58	7.5	0.75	8	9.5	0.56
White collar parent	695	17.3	112	14.5	0.060	10	12.2	0.23
Older siblings	1980	48.4	389	50.0	0.42	37	44.1	0.43

E-Figure legends

Figure E1. Venn diagram showing the study population of 862 participants (indicated in pink) derived from the BAMSE cohort (N=4089): A subgroup of population-based participants (N=778) enriched with peanut extract sensitized participants (N=84) from the group of eligible participants (N=1699).

Figure E2. Ara h 2/Ara h 6 sensitization overlap at 4, 8 and 16 years of age. Median IgE levels (ISU-E) in the sensitization subgroups. N=862 (peanut-enriched study group)

Figure E3. Ara h 1/Ara h 2/Ara h 3 sensitization overlap at 4, 8 and 16 years of age. Median IgE levels (ISU-E) in the sensitization subgroups. N=862 (peanut-enriched study group)

Figure E4. Likelihood of reporting symptoms to peanut at 16 years of age in relation to **A)** Ara h 2 IgE level at 4 years of age (ISU-E), **B)** Ara h 2 IgE level at 4 years of age (ISU-E) (incident symptoms), **C)** peanut extract IgE level at 4 years of age (kU_A/L) or **D)** number of sensitizing peanut allergen molecules (Ara h 8 excluded)/ peanut extract sensitization at 4 years of age. N=862 (peanut-enriched study group)