

ORIGINAL ARTICLE

Epidemiology of Allergic Disease

Environmental peanut exposure increases the risk of peanut sensitization in high-risk children

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Summary

Background: High household peanut consumption is associated with the development of peanut allergy, especially when peanut allergic cases are compared against atopic controls; thus, environmental peanut exposure (EPE) may be a risk factor for peanut sensitization and allergy. In this study, we explored the relationship between EPE and school-age peanut sensitization in a population-based cohort.

Methods: Maternal bed dust was collected postnatally, and EPE was quantified using a polyclonal peanut ELISA. Peanut sensitization was assessed by specific IgE to peanut extract and sIgE to peanut protein component allergens Ara h 1, 2 or 3 ≥ 0.35 kU/L (primary peanut sensitization). Initial nested case-control analysis was performed comparing peanut-sensitized cases against high-risk controls (matched for parental atopy) ($n = 411$) using a conditional regression analysis. This was followed by whole cohort analysis ($n = 1878$) comparing EPE against peanut sIgE sensitization at ages 4 and 8 years using generalized estimating equations and against primary peanut sensitization at age 8 years using a logistic regression model. Finally, a subgroup analysis was performed comparing the impact of EPE in peanut-sensitized vs egg-sensitized, peanut-tolerant individuals using logistic regression analysis. Levels of EPE were compared between groups using the Mann-Whitney U test.

Results: In the nested case-control analysis, a higher level of EPE around birth was associated with peanut-specific IgE sensitization at age 4 years (OR=1.41, 95% CI:1.05-1.90) and primary peanut sensitization at age 8 years (OR=2.11, 95% CI:1.38-3.22) compared against high-risk controls. When the whole BAMSE cohort was assessed, EPE was no longer associated with peanut sensitization; however, on subgroup analysis, EPE was associated with primary peanut sensitization when compared against egg-sensitized peanut-tolerant controls with an adjusted odds ratio of 1.44 per unit EPE (95% CI:1.06-1.94). There was no significant interaction between EPE and *FLG* loss-of-function mutations, egg sensitization at age 4 years, infantile eczema or parental atopy on peanut sensitization.

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Conclusions: Higher levels of environmental exposure to peanut in the first few months of life appear to increase the probability of developing school-age peanut sensitization in atopic children (based on egg sensitization and parental atopy).

KEYWORDS

BAMSE, egg sensitization, environmental peanut exposure, *FLG* mutation, infantile eczema peanut sensitization

1 | INTRODUCTION

Peanut allergy has increased,^{1,2} is a leading cause of anaphylaxis in food allergy³ and has a significant impact on quality of life for the child and their family.^{4,5} Most children react on first known oral exposure to peanut; thus, sensitization must be occurring earlier.^{6,7} Understanding the way children become sensitized to peanut is therefore imperative to prevent this condition. Observational⁸ and animal work^{9,10} suggests that epicutaneous peanut exposure and early-onset severe eczema may play an important role in peanut sensitization. Epicutaneous exposure may be the route that peanut levels in dust sensitize a child in early life, particularly if the skin barrier is broken through eczema or specific genetic mutations associated with skin barrier dysfunction (eg filaggrin (*FLG*) mutations or eczema). We have recently shown in the Manchester Asthma and Allergy Study (MAAS) and Consortium of Food Allergy Research (CoFAR) cohort that *FLG* loss-of-function mutations and eczema severity, respectively, can increase the impact of early EPE on peanut sensitization and allergy.^{11,12}

A dose-response relationship has also been demonstrated between household peanut consumption (used as an indirect marker for environmental peanut exposure: EPE) and the risk of developing peanut allergy in young children.¹³ Fox et al compared household peanut consumption in peanut allergic individuals against children who were at high risk of developing peanut allergy (due to the presence of egg allergy) but had not developed peanut allergy. Household peanut consumption was ten times higher in households with infants with peanut allergy vs children with egg allergy without peanut allergy but was only 3 times higher in non-atopic controls, highlighting that household peanut consumption was more likely to be associated with peanut allergy in high-risk children.¹³ Household peanut consumption and peanut sensitization have also been

associated in other studies.¹⁴ Peanut antigen in the infant's bed dust and play-area-dust is highly positively correlated with household peanut consumption and stimulates basophils from peanut allergic children, thus has the potential to also sensitize individuals.¹⁵

Peanut sensitization (specific IgE $\geq$ 0.35kU/L) is present in up to 10% of children, and protein allergen molecule analysis of the seed storage proteins in peanut (Ara h 1, 2 and 3) has been shown to improve the specificity and sensitivity of diagnosing peanut allergy.^{16,17} In BAMSE, children with IgE reactivity to Ara h 1, 2 or 3 at age 8 years (but not Ara h 8) reported peanut allergic symptoms in 87% of cases, whereas children with IgE reactivity exclusively to Ara h 8 reported peanut allergic symptoms in only 17% of cases which were also milder.¹⁸ Sweden has high levels of birch pollen sensitization which explains the Bet v 1 in-vitro cross-reactivity with peanut Ara h 8;¹⁸⁻²⁰ thus, in this study, we differentiated between primary peanut sensitization (IgE reactivity to Ara h 1, 2 or 3) and pollen cross-reactivity (IgE reactivity to Ara h 8).

This study aimed to assess whether EPE (as defined by peanut protein levels in household dust) is a risk factor for the development of school-age peanut sensitization. The analysis was carried out in two steps for financial and logistical reasons; firstly among high-risk children (with parental atopy matched to peanut sensitized cases), in a nested cases analysis and secondly among the whole studied population (a cohort analysis). Given previous findings, we also wanted to ascertain the modifying effects of *FLG* loss-of-function mutations, infantile eczema and preceding egg sensitization.

2 | METHODS

The BAMSE study is an unselected Swedish population birth cohort. The design and methodology are described elsewhere.²¹ In brief,

4089 unselected newborn children were recruited from 1994 to 1996 and have been evaluated for various health conditions over time. No intervention was performed. The BAMSE study obtained ethical approval for environmental sampling of dust.

2.1 | Demographics

Serum-specific IgE to peanut (ImmunoCAP system, ThermoFisher, Sweden) was measured at 4 and 8 years, and children were defined as "peanut sIgE-sensitized" if peanut-specific IgE was ≥ 0.35 kU/L. Peanut protein component allergens Ara h 1, 2, 3, 8 and 9 were assessed at 8 years (ImmunoCAP system, ThermoFisher, Sweden) in all children sensitized to peanut at age 8 years, irrespective of sensitization to birch. In addition, those children who were peanut-sensitized at age 4 years but lost their sensitization at age 8 were also analysed for these allergens. All other children with negative peanut sIgE at age 8 years were assumed to have negative component allergens to peanut. Children were considered to have primary peanut sensitization if Ara h 1, 2 or 3 was ≥ 0.35 kU/L, and no primary peanut sensitization if Ara h 1, 2 and 3 were < 0.35 kU/L or peanut sIgE was < 0.35 kU/L at 8 years (where no sIgE peanut protein component allergens were available). Twenty-four (1.3%) children with positive peanut-specific IgE at 8 years of age but with no available serum for peanut protein component allergen analysis were excluded from this analysis. Egg sensitization was defined as egg-specific IgE ≥ 0.35 kU/L at 4 years of age. Previous allergic reactions to a food including itching in the mouth, nose/eye problems, trouble breathing, vomiting or diarrhoea, eczema or nettle rash were used from parental questionnaires at age 8 years.

Parental atopy was defined as a doctor's diagnosis of asthma and prescription of asthma medication and/or a doctor's diagnosis of hayfever in combination with furred pets and/or pollen allergy at the time of questionnaire. This definition of parental atopy was selected rather than the same definition with inclusion of a doctor's diagnosis of eczema (excluding contact dermatitis), because the children of parents with atopy including eczema were less atopic (lower infantile eczema), and parental atopy including eczema was not associated with the outcome of interest (primary peanut sensitization at 8 years of age); this is probably because adult eczema is often non-atopic. Genotyping was performed for *FLG* mutations common in Scandinavia using TaqMan allelic discrimination assays for R501X and R2447X and matrix-assisted laser desorption/ionization time-of-flight mass spectrometry for 2282del4. Children with a *FLG* mutation in any of these positions were classified as having a *FLG* loss-of-function mutation.

A reported history of infantile eczema was assessed by a validated eczema questionnaire with sensitivity of 92% and specificity of 100%.²² Eczema was defined as dry skin, itchy rashes for 2 weeks or more and age-specific localization (face or arms/legs extension surfaces or arms/legs flexures or wrists/ankles flexures) and/or doctor's diagnosis of eczema after 3 months and up to 1 year of age. Severity of eczema was assessed from questionnaires using three different measures:

1. Extent of localization of eczema (up to 10 areas) at or before their one-year assessment
2. Topical steroids used as treatment for an itchy rash in the last year at their two-year assessment (not available at their one-year assessment)
3. Persistent eczema at age 8 years (using the same definition as above).

Ethnicity was based on where the parents were born. Allergic rhinitis at 4 and 8 years was assessed using International Study of Asthma and Allergies in Childhood (ISAAC) validated questionnaires, and was defined as persistent rhinitis without a common cold in the last 12 months before 4 and 8 years, respectively.²³

2.2 | Dust collection methodology

Dust samples were collected at a median of 2 months of age from the mother's mattress. The mother's mattress was vacuumed for 2.5 minutes with a small disposable filter bag (Allergy Control Products Inc. Ridgefield CT, USA) inserted in the front hose of the vacuum. The dust-containing filter bags were sealed in plastic bags and stored at -20°C . Dust samples were sieved, and fine dust was weighed and extracted in proportional volumes of extraction solution as previously described.²⁴ Peanut protein in dust was determined using the Veratox polyclonal ELISA against whole peanut protein (Neogen Corporation, Lansing, MI, USA). The lower limit of quantitation (LLQ) of the assay was defined as 100 ng/mL, and samples below this value were defined as 50 ng/mL (LLQ/2).²⁵ Results were converted from ng/mL into μg peanut protein/g dust. Due to batch-to-batch variability of the Veratox ELISA, results were batch corrected prior to being entered into the final statistical analysis.

2.3 | Statistical analysis

Data were entered into an SPSS (SPSS 19.0; SPSS Inc, Chicago, IL, USA) and STATA spreadsheet (Timberlake Consultants Ltd London, UK) for the purpose of analysis. Peanut protein levels in dust ($\mu\text{g/g}$) underwent natural log transformation. Initial nested case-control analysis was performed with peanut sIgE-sensitized cases at 4 years of age-matched for sex and parental atopy with a 2:1 control ($n = 274$) to case ($n = 137$) matching. Children with primary peanut sensitization defined by Ara h 1, 2 or 3 ≥ 0.35 kU/L at 8 years of age were matched against controls at age 8 years with the same sex and level of parental atopy with a 2:1 ratio of controls ($n = 130$) to cases ($n = 65$). Given that these controls were matched for the higher levels of parental atopy in the primary peanut-sensitized cases, these controls were deemed to be high-risk as the primary peanut-sensitized cases had higher levels of parental atopy than the population overall (46.7% vs 32.6%). Conditional logistic regression (incorporating matching) was performed for the case-control analysis using robust standard error for peanut sIgE sensitization at 4 years and primary peanut sensitization at age 8 years.

Subsequently, analysis was performed for the relationship between early EPE and peanut sensitization in all children from the BAMSE cohort with available postnatal maternal bed dust and *FLG* genotyping ($n = 1878$). Logistic regression analysis was performed for primary peanut sensitization at 8 years of age. Factors associated with peanut sIgE sensitization were assessed using generalized estimating equations (GEE) with an exchangeable working correlation matrix to account for repeated measures within individuals at 4 and 8 years. Univariate followed by multivariate regression analysis was performed including EPE plus other covariates significantly associated with peanut sensitization ($P \leq .05$) which also improved the quality of fit of the multivariate model using the Akaike information criterion (AIC) for logistic regression and the quasi-likelihood independence models criterion (QIC) for GEE analyses.

Where the *FLG* mutation covariate was entered into the statistical model, participants with missing ethnicity data ($35/1878 = 1.9\%$) and non-Caucasians ($165/1842 = 8.8\%$) were excluded as distinct *FLG* loss-of-function mutations are present in different populations, and the *FLG* loss-of-function mutations assessed in this study have only been associated with eczema in Caucasian European populations.^{26,27} Peanut levels in dust ($\mu\text{g/g}$) were compared between groups using the Mann-Whitney U test. Statistical significance was assessed at $P < .05$.

3 | RESULTS

Details of demographics and clinical characteristics included in this study are described in Table S1. Median peanut protein [IQR] in dust was $4.07 \mu\text{g/g}$ [1.58, 11.86]. Peanut extract sIgE sensitization was 6.6% at 4 years ($n = 103/1572$) and 8.6% at 8 years ($n = 161/1876$). Primary peanut sensitization at 8 years was 4.1% ($n = 75/1854$), of which 74/75 (99%) had Ara h 2 $\geq 0.35\text{kU/L}$.

TABLE 1 Univariate conditional logistic regression incorporating matching for peanut-sensitized cases vs non-sensitized controls matched for sex and parental atopy. Peanut sIgE sensitization ($\geq 0.35\text{kU/L}$) at age 4 y and primary peanut sensitization (Ara h 1, 2 or 3 $\geq 0.35\text{kU/L}$) at age 8 y

	Peanut sIgE sensitized at age 4 y ($n = 411$)			Primary peanut sensitization sensitized at age 8 y ($n = 195$)		
	OR	95% CI	P value	OR	95% CI	P-value
EPE ($\mu\text{g/g}$ ln transformed)	1.23	1.06-1.43	<.01	1.29	1.04-1.61	.02
Number of biological siblings	1.38	0.63-3.03	.42	2.50	0.77-8.16	.13
≥ 1 <i>FLG</i> loss-of-function mutation (excluding non-Caucasians)	2.67	1.18-6.04	.02	3.00	0.84-10.75	.09
History of infantile eczema	7.35	4.20-12.83	<.001	6.39	2.95-13.83	<.001
Egg sIgE sensitization at 4 y	18.91	7.50-47.66	<.001	12.85	3.78-43.67	<.001
^a Allergic rhinitis at 4 y (ISAAC)	3.44	1.90-6.23	<.001	2.53	1.04-6.18	.04
^a Allergic rhinitis at 8 y (ISAAC)	11.44	5.68-23.07	<.001	12.97	4.54-37.05	<.001
Exclusively breastfed (months)	0.95	0.88-1.03	.22	0.98	0.87-1.11	.77
Breastfed (excl/partial) (months)	0.99	0.93-1.05	.74	1.01	0.93-1.11	.76
Maternal age at baseline (in years)	0.99	0.94-1.04	.65	1.05	0.99-1.12	.12

^aAllergic rhinitis was included to assess whether false-positive results for peanut sensitization were being found due to cross-reactivity with grass or tree pollens.

3.1 | Nested case-control analysis for peanut sIgE and primary peanut sensitization

We compared peanut-sensitized children against children without peanut sensitization matched for sex and parental atopy as described in the statistical methods. EPE was a risk factor for both peanut extract sIgE sensitization at 4 years and primary peanut sensitization at 8 years with a 23% and 29% increased odds of developing peanut sensitization per natural log (ln) unit increase in EPE, respectively (Table 1). In children who were peanut extract sIgE-sensitized but not sensitized to peanut protein component allergens Ara h 1, 2 and/or 3, there was only a trend towards a significant association between EPE and peanut sensitization (OR=1.20, 95% CI: 0.97-1.48).

Differential relationship between EPE and primary peanut sensitization and non-clinically significant peanut sensitization is displayed in Figure 1. Median peanut protein in dust was higher in peanut sIgE-sensitized children ($3.39 \mu\text{g/g}$, IQR 1.41-11.01, $n = 137$) than non-sensitized controls ($2.28 \mu\text{g/g}$, IQR 0.88-5.14, $n = 274$) ($P < .01$); and higher in primary peanut-sensitized children ($4.64 \mu\text{g/g}$, IQR 1.58-12.77, $n = 65$) than in children that were not sensitized to Ara h 1, 2 or 3 ($2.24 \mu\text{g/g}$, IQR 0.88-4.74, $n = 130$) ($P < .01$).

On multivariate conditional logistic regression analysis, EPE was significantly associated with peanut-specific IgE and primary peanut sensitization with a 1.41- and 2.1-fold increase in odds of developing peanut sensitization per unit increase EPE, respectively (Table 2). Infantile eczema and egg sensitization were both significantly associated with sensitization to peanut protein component allergens Ara h 1, 2 and/or 3 (Table 2). *FLG* loss-of-function mutations increased the probability of developing peanut-specific IgE sensitization 3.78-fold and primary peanut sensitization 7.33-fold. Allergic rhinitis at age 8 years was associated with both the probability of developing peanut-specific IgE sensitization at age 4 years and primary peanut sensitization at age 8 years in the multivariate analysis. The number of

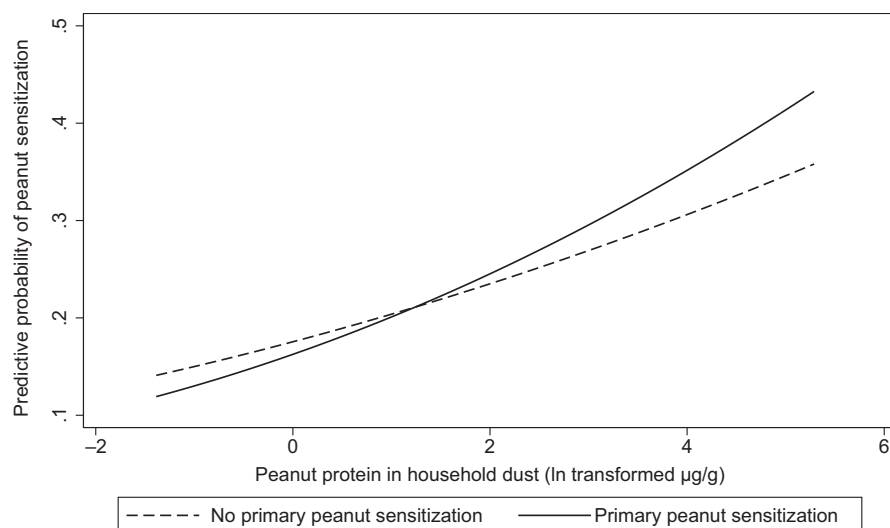


FIGURE 1 Mean predictive probability of peanut sensitization in children with primary peanut sensitization (Ara h 1, 2 or 3 ≥ 0.35 kU/L) vs children with peanut-specific IgE ≥ 0.35 kU/L but with negative results for primary peanut (Ara h 1, 2 or 3 < 0.35 kU/L)

biological siblings, duration of exclusive and total breastfeeding and maternal age at baseline were not associated with peanut sensitization. There was no significant interaction between EPE and either infantile eczema, egg sensitization or *FLG* loss-of-function mutations on primary peanut sensitization (data not shown).

3.2 | Whole cohort analysis for peanut extract sIgE sensitization and primary peanut sensitization

In a second step, following positive findings obtained on case-control analysis, we assessed the relationship between early EPE and peanut sensitization in all children from the BAMSE cohort with available postnatal maternal bed dust and *FLG* genotyping ($n = 1878$). On both univariate (Table S2) and multivariate analyses (Table 3), infantile eczema, egg sensitization at age 4 years, tree nut and soya allergy at age 8 years, *FLG* loss-of-function mutation and allergic rhinitis at age 8 years were associated with the probability of peanut sIgE sensitization at age 4 and 8 years. For primary peanut sensitization at age 8, significant associations were also seen on univariate (Table S2) and multivariate analyses (Table 3) for infantile eczema, egg sensitization, tree nut and soya allergy at age 8 years and allergic rhinitis at age 8 years. Although measures of eczema severity and several other food allergies at age 8 years were significantly associated with peanut sensitization on univariate analysis, these variables were no longer significantly associated in the multivariate analysis. EPE was not associated with peanut sIgE sensitization or primary peanut sensitization. There was no interaction between EPE and *FLG* mutations, infantile eczema, egg sensitization, allergic reaction to tree nut or soya by age 8 years, or parental atopy on peanut sensitization.

3.3 | Subgroup analysis of primary peanut sensitization against egg-sensitized non-peanut allergic individuals

On subgroup analysis, we compared primary peanut-sensitized children ($n = 75$) against children who were sensitized to egg (sIgE ≥ 0.35 kU/L

at age 4 years) but did not go on to have primary peanut sensitization at age 8 years ($n = 59$). EPE increased the probability of primary peanut sensitization on both univariate (OR=1.33 95% CI:1.01-1.74, $P < .05$, $n = 134$) (Table S3) and multivariate analyses (OR=1.44, 95% CI:1.06-1.94, $P=0.018$, $n = 133$, AIC: 167) adjusting for infantile eczema and allergic reaction to soya by age 8 years. Peanut levels in household dust were also significantly higher in primary peanut-sensitized children (median 4.79 $\mu\text{g/g}$, IQR 1.63-12.00) vs egg-sensitized, non-primary peanut-sensitized children (median 2.35 $\mu\text{g/g}$, IQR 1.23-6.15) ($P = .03$). We assessed the impact of EPE on peanut sensitization when using other high-risk groups of children who were not peanut-sensitized at age 8 years (*FLG* mutations, infantile eczema, soya allergy and parental atopy), and there was no significant association.

4 | DISCUSSION

In the prospective birth cohort BAMSE, there was an exposure-response relationship between EPE (quantified using peanut protein levels from maternal mattress dust postnatally) and primary peanut sensitization (Ara h 1, 2 or 3 ≥ 0.35 kU/L) among high-risk children (in a nested cases analysis). When all children in the BAMSE cohort with available dust and *FLG* status were assessed, there was no association between EPE and peanut sensitization; however, on subgroup analysis, there was an exposure-response relationship between EPE and primary peanut sensitization when compared against high-risk egg-sensitized children. In the nested cases analysis, the exposure-response relationship was stronger between EPE and primary peanut sensitization (Ara h 1, 2 or 3 ≥ 0.35 kU/L) than between EPE and non-clinically relevant peanut sensitization (peanut sIgE ≥ 0.35 kU/L but negative results for Ara h 1, 2 or 3 < 0.35 kU/L) (Figure 1). This supports the concept that EPE increases the probability of developing clinically relevant peanut sensitization which is more likely to develop into peanut allergy rather than non-specific peanut sensitization.

In other cohorts, we have shown a synergistic effect between EPE and markers of an impaired skin barrier (infantile eczema and

TABLE 2 Multivariate conditional logistic regression incorporating peanut-sensitized cases vs non-sensitized controls matched for sex and parental atopy. Peanut sIgE sensitization (≥ 0.35 kU/L) at age 4 years and primary peanut sensitization (Ara h 1, 2 or 3 ≥ 0.35 kU/L) at age 8 years. Non-Caucasians were excluded due to inclusion of *FLG* mutations as a covariate

	Peanut sIgE sensitized at age 4 y (excluding non-Caucasians) (n = 237) ^a AIC: 90.78			Primary peanut sensitization at age 8 y (excluding non-Caucasians) (n = 143) ^a AIC: 49.19		
	OR	95% CI	P Value	OR	95% CI	P Value
EPE (μ g/g In transformed)	1.41	1.05-1.90	.02	2.11	1.38-3.22	.001
≥ 1 <i>FLG</i> loss-of-function mutation	3.78	0.93-15.36	.06	7.33	1.21-44.21	.03
History of infantile eczema	2.53	0.98-6.51	.05	3.93	1.03-15.00	.05
Egg sIgE sensitization: 4 y	28.00	5.24-149.39	<.001	39.9	1.47-1081.68	.03
^b Allergic rhinitis: 8 y (ISAAC)	12.57	4.164-37.98	<.001	28.93	3.77-221.61	<.001

^aSmaller AIC is better.

^bAllergic rhinitis at age 8 y was selected as it improved the fit of the model (AIC better than allergic rhinitis at age 4 y).

TABLE 3 Multivariate analysis of peanut-specific IgE sensitization aged 4 and 8 y using generalized estimating equations (GEE) to account for repeated measures within individuals at 4 and 8 y and logistic regression analysis for primary peanut sensitization at age 8 y in the BAMSE cohort, goodness of fit assessed by QIC and AIC

	Multivariate peanut sIgE sensitization (excluding non-Caucasians) (n = 1367) QIC:935			Multivariate primary peanut sensitization (n = 1521) AIC:386		
	OR	95% CI	P Value	OR	95% CI	P Value
EPE (μ g/g In transformed)	0.94	0.80-1.11	.45	1.08	0.87-1.34	.48
≥ 1 <i>FLG</i> loss-of-function mutations	2.52	1.24-5.10	.01	ns ^a		
History of infantile eczema	2.75	1.67-4.48	<.001	4.02	2.14-7.54	<.001
Tree nut allergic reaction by age 8 y	9.30	4.94-17.53	<.001	4.80	2.38-9.66	<.001
Soya allergic reaction by age 8 y	18.21	4.75-69.80	<.001	2.54	3.85-42.07	<.001
Egg sIgE sensitization at 4 y	7.66	3.92-14.95	<.001	4.24	1.98-9.09	<.001
Allergic rhinitis aged 8 y (ISAAC)	4.02	2.49-6.52	<.001	2.01	1.05-3.83	.034

^ans: not significant on multivariate analysis therefore not included in final multivariate analysis.

FLG loss-of-function mutations) on peanut sensitization and allergy.^{11,12} This follows the hypothesis that children with an inflamed disrupted skin barrier may become sensitized to food proteins in their environment via transcutaneous sensitization.⁸ Although in BAMSE we did not find significant interactions between EPE and *FLG* mutations or infantile eczema on peanut sensitization, we did show on subgroup analysis that early EPE has more impact on the probability of developing peanut sensitization when compared against high-risk children (defined by parental atopy in the nested cases analysis and egg sensitization in the whole cohort analysis) than in children without these risk factors.

The BAMSE cohort is relatively non-atopic, being from the general population in Sweden, which may have contributed to lower strength of associations between EPE and peanut sensitization overall, and in other groups such as children with a history of eczema or filaggrin mutations. We did find an association between EPE and peanut sensitization when we compared primary peanut-sensitized children against children who were sensitized to egg but did not go on to have primary peanut sensitization. We postulated that children with egg sensitization who went on to develop peanut sensitization were probably exposed to a higher levels of EPE than those who did not go on to develop peanut sensitization as this reflected the findings by Fox et al who showed that household peanut consumption

during the first year of life (as an indirect marker of EPE) was higher in peanut allergic cases than in egg-allergic controls.¹³

Egg sensitization was highly associated with peanut sensitization in the BAMSE study, which has previously been shown in numerous other cohort studies;^{11,12,28} it may be that egg sensitization shows a predisposition to mount allergic antibodies to other allergens as egg allergy is highly associated with the development of other food allergies, asthma and aeroallergen sensitization.^{29,30} Observational studies have shown that in children with severe, early-onset eczema, up to 50% are sensitized to egg;³¹ thus, egg sensitization may be an additional marker of eczema severity and predisposition to transcutaneous sensitization. In fact, of children who were egg-sensitized at 4 years in BAMSE, there was a higher use of early topical steroids for eczema treatment (85.4% vs 69.5%) than in children without egg sensitization.

We also considered other factors that may have influenced the differences found between the effects of EPE on peanut sensitization in BAMSE study vs other cohorts such as the level of peanut protein found in dust. The median level of peanut protein in maternal bed dust in the BAMSE cohort was 4.07 μ g/g [IQR 1.58, 11.86], similar to that found in another UK study by our group (median 4.19 IQR 0.54-24.89).²⁴ The level of peanut in dust was higher in a US study that measured peanut from the living room floor (median 39.1 μ g/g IQR 0.4-1.33).¹⁰ It would, however, be expected that

peanut levels would be higher in America due to higher levels of peanut consumption.

Infantile eczema was one of the most important risk factors for peanut sensitization in BAMSE; this association has been cited in numerous studies.^{8,31} Measures of eczema severity (extent of localization, topical steroid use and persistent eczema) were associated with peanut sensitization on univariate analysis but were no longer associated with multivariate analysis. Eczema severity has previous been shown to be an independent risk factor for food sensitization in the EAT (Enquiring About Tolerance) study.³² The EAT study used the SCORAD eczema severity scoring system; however, this could not be performed in the BAMSE study as the evaluations at 1, 2, 4 and 8 years were based on parental questionnaires.

FLG loss-of-function mutations were less important than infantile eczema in predicting the development of peanut sensitization, and FLG mutations became more important if infantile eczema was not included in the regression model. Previous studies have found that children that carry a FLG mutation have an increased risk of peanut allergy even after adjusting for eczema.³³ In this study, eczema seemed to be the overriding factor for peanut sensitization; however, it should be noted that FLG mutations were quite low in BAMSE (7.14%) in comparison with other population-based cohorts.^{26,33,34} Tree nut and/or soya allergy by age 8 was also associated with primary peanut sensitization at age 8 years; co-existent peanut and tree nut allergy have been shown in numerous studies,^{35,36} whereas co-existent soya and peanut allergy are reportedly low in other populations.³⁷

Strength of the present study includes the population-based design and relatively high number of study participants. Limitations of this study included the lack of diagnostic food challenges to determine peanut allergy as the BAMSE study was not originally designed to evaluate peanut allergic outcomes; however, primary peanut sensitization has been shown to be a much more accurate predictor of clinically confirmed peanut allergy than peanut-specific IgE.^{38,39} Certain potential confounders were not available such as parental peanut allergy or other parental food allergies, maternal peanut consumption during pregnancy and breastfeeding and infant peanut consumption. Therefore, we are unable to assess whether infant peanut consumption could have protected the children against environmental exposure to peanut as has been shown in previous studies;^{13,35} however, when BAMSE was recruiting participants in 1996, the number of infants eating peanut was very low. Caucasian ethnicity was based on place of parental birth rather than self-reported ethnicity; however, when the BAMSE study was recruiting participants 1994-1996, most parents born in Sweden or Northern Europe would have been of Caucasian descent.⁴⁰ Findings on case-control analysis could not be replicated on whole cohort analysis; therefore, these findings need to be confirmed in other population-based cohorts.

5 | CONCLUSION

In summary, although EPE did not increase the risk of peanut sensitization in children who did not already have atopic risk factors, the

findings from the BAMSE cohort support the hypothesis that in specific high-risk groups, peanut levels in dust around the time of birth may pose a risk for later peanut sensitization. Further prospective studies are needed to confirm these findings in other populations.

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CONFLICT OF INTERESTS

H. A. Brough has received research support from Food allergy & Research Education (FARE), Action Medical Research and has received research support, lecture fees and travel support from ThermoFisher Scientific. G. Lack has received research support from National Institute of Allergy and Infectious Diseases (NIAID, NIH), Food Allergy & Research Education (FARE), MRC and Asthma UK Centre, UK Department of Health through NIHR Biomedical Research Centre Award, National Peanut Board (NPB), Food Standards Agency (FSA), Action Medical Research, and has stock/stock options with DBV Technologies. V. Turcanu have received research support from the National Peanut Board. I Kull, K Makinson, E Hallner, C Söderhäll, A Douiri, M Penagos, E Melén, A Bergström and M Wickman declare that they have no relevant conflict of interests.

AUTHOR CONTRIBUTIONS

HB, IK, EH, MW and GL had substantial contributions to the conception and design of the study. HB, KR, MP and VR made substantial contributions to acquisition of peanut dust data and analysis. HB and AD performed the statistical analysis of results. IK, EH, CS, AB, EM and MW had substantial contribution to the BAMSE study and obtaining data to perform this study. CS performed the FLG genotyping. All authors contributed to the drafting and revising the manuscript for intellectual content and have approved the version to be published.

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REFERENCES

1. Venter C, Hasan AS, Grundy J, et al. Time trends in the prevalence of peanut allergy: three cohorts of children from the same geographical location in the UK. *Allergy*. 2010;65:103-108.
2. Sicherer SH, Munoz-Furlong A, Godbold JH, Sampson HA. US prevalence of self-reported peanut, tree nut, and sesame allergy: 11-year follow-up. *J Allergy Clin Immunol*. 2010;125:1322-1326.
3. Bock SA, Munoz-Furlong A, Sampson HA. Further fatalities caused by anaphylactic reactions to food, 2001-2006. *J Allergy Clin Immunol*. 2007;119:1016-1018.

4. Avery NJ, King RM, Knight S, Hourihane JOB. Assessment of quality of life in children with peanut allergy. *Pediatr Allergy Immunol*. 2003;14:378-382.
5. King RM, Knibb RC, Hourihane JO. Impact of peanut allergy on quality of life, stress and anxiety in the family. *Allergy*. 2009;64:461-468.
6. Hourihane JOB, Kilburn SA, Dean P, Warner JO. Clinical characteristics of peanut allergy. *Clin Exp Allergy*. 1997;27:634-639.
7. Monti G, Muratore MC, Peltran A, et al. High incidence of adverse reactions to egg challenge on first known exposure in young atopic dermatitis children: predictive value of skin prick test and radioallergen sorbent test to egg proteins. *Clin Exp Allergy*. 2002;32:1515-1519.
8. Lack G, Fox D, Northstone K, Golding J. Team ALSoPaCS. Factors associated with the development of peanut allergy in childhood. *N Engl J Med*. 2003;348:977-985.
9. Strid J, Hourihane J, Kimber I, Callard R, Strobel S. Disruption of the stratum corneum allows potent epicutaneous immunization with protein antigens resulting in a dominant systemic Th2 response. *Eur J Immunol*. 2004;34:2100-2109.
10. Reynolds LA, Finlay BB. Early life factors that affect allergy development. *Nat Rev Immunol*. 2017;17:518-528.
11. Brough HA, Simpson A, Makinson K, et al. Peanut allergy: effect of environmental peanut exposure in children with filaggrin loss-of-function mutations. *J Allergy Clin Immunol*. 2014;134:867-875 e1.
12. Brough HA, Liu AH, Sicherer S, et al. Atopic dermatitis increases the effect of exposure to peanut antigen in dust on peanut sensitization and likely peanut allergy. *J Allergy Clin Immunol*. 2015;135:164-170.
13. Fox AT, Sasieni P, Du Toit G, Syed H, Lack G. Household peanut consumption as a risk factor for the development of peanut allergy. *J Allergy Clin Immunol*. 2009;123:417-423.
14. Garcia-Boyano M, Pedrosa M, Quirce S, Boyano-Martinez T. Household almond and peanut consumption is related to the development of sensitization in young children. *J Allergy Clin Immunol*. 2016;137:1248-1251.
15. Brough HA, Santos A, Makinson K, et al. Peanut protein in household dust is related to household peanut consumption and is biologically active. *J Allergy Clin Immunol*. 2013;132:630-638.
16. Nicolaou N, Murray C, Belgrave D, Poorafshar M, Simpson A, Custovic A. Quantification of specific IgE to whole peanut extract and peanut components in prediction of peanut allergy. *J Allergy Clin Immunol*. 2011;127:684-685.
17. Dang TD, Tang M, Choo S, et al. Increasing the accuracy of peanut allergy diagnosis by using Ara h 2. *J Allergy Clin Immunol*. 2012;129:1056-1063.
18. Asarjoj A, Ostblom E, Kull I, et al. Sensitization to inhalant allergens between 4 and 8 years of age is a dynamic process: results from the BAMSE birth cohort. *Clin Exp Allergy*. 2008;38:1507-1513.
19. Asarjoj A, Ostblom E, Ahlstedt S, et al. Reported symptoms to peanut between 4 and 8 years among children sensitized to peanut and birch pollen - results from the BAMSE birth cohort. *Allergy*. 2010;65:213-219.
20. Asarjoj A, Hamsten C, Lupinek C, et al. Prediction of peanut allergy in adolescence by early childhood storage protein-specific IgE signatures: the BAMSE population-based birth cohort. *J Allergy Clin Immunol*. 2017;140:587-590.e7.
21. Wickman M, Kull I, Pershagen G, Nordvall SL. The BAMSE project: presentation of a prospective longitudinal birth cohort study. *Pediatr Allergy Immunol*. 2002;13 Suppl 15:11-13.
22. Bohme M, Lannero E, Wickman M, Nordvall SL, Wahlgren CF. Atopic dermatitis and concomitant disease patterns in children up to two years of age. *Acta Derm Venereol*. 2002;82:98-103.
23. Strachan D, Sibbald B, Weiland S, et al. Worldwide variations in prevalence of symptoms of allergic rhinoconjunctivitis in children: the International Study of Asthma and Allergies in Childhood (ISAAC). *Pediatr Allergy Immunol*. 1997;8:161-176.
24. Brough HA, Makinson K, Penagos M, et al. Distribution of peanut protein in the home environment. *J Allergy Clin Immunol*. 2013;132:623-629.
25. Rw H, Ld R. Estimation of average concentration in the presence of nondetectable values. *App Occup Environ Hyg*. 1990;5:46-51.
26. Baurecht H, Irvine AD, Novak N, et al. Toward a major risk factor for atopic eczema: meta-analysis of filaggrin polymorphism data. *J Allergy Clin Immunol*. 2007;120:1406-1412.
27. Weidinger S, O'Sullivan M, Illig T, et al. Filaggrin mutations, atopic eczema, hay fever, and asthma in children. *J Allergy Clin Immunol*. 2008;121:1203-1209.
28. Du Toit G, Roberts G, Sayre PH, et al. Identifying infants at high risk of peanut allergy: the Learning Early About Peanut Allergy (LEAP) screening study. *J Allergy Clin Immunol*. 2013;131:135-143 e1-e12.
29. Tariq SM, Matthews SM, Hakim EA, Arshad SH. Egg allergy in infancy predicts respiratory allergic disease by 4 years of age. *Pediatr Allergy Immunol*. 2000;11:162-167.
30. Gaffin JM, Sheehan WJ, Morrill J, et al. Tree nut allergy, egg allergy, and asthma in children. *Clin Pediatr*. 2011;50:133-139.
31. Hill DJ, Hosking CS, Oranje AP, et al. Confirmation of the association between high levels of immunoglobulin E food sensitization and eczema in infancy: an international study. *Clin Exp Allergy*. 2008;38:161-168.
32. Flohr C, Perkin M, Logan K, et al. Atopic dermatitis and disease severity are the main risk factors for food sensitization in exclusively breastfed infants. *J Invest Dermatol*. 2014;134:345-350.
33. Brown SJ, Asai Y, Cordell HJ, et al. Loss-of-function variants in the filaggrin gene are a significant risk factor for peanut allergy. *J Allergy Clin Immunol*. 2011;127:661-667.
34. Johansson EK, Bergstrom A, Kull I, et al. IgE sensitization in relation to preschool eczema and filaggrin mutation. *J Allergy Clin Immunol*. 2017;140:1572-1579.e5.
35. Du Toit G, Katz Y, Sasieni P, et al. Early consumption of peanuts in infancy is associated with a low prevalence of peanut allergy. *J Allergy Clin Immunol*. 2008;122:984-991.
36. Sicherer SH, Munoz-Furlong A, Sampson HA. Prevalence of peanut and tree nut allergy in the United States determined by means of a random digit dial telephone survey: a 5-year follow-up study. *J Allergy Clin Immunol*. 2003;112:1203-1207.
37. Sampson HA, McCaskill CC. Food hypersensitivity and atopic dermatitis: evaluation of 113 patients. *J Pediatr*. 1985;107:669-675.
38. Nicolaou N, Poorafshar M, Murray C, et al. Allergy or tolerance in children sensitized to peanut: prevalence and differentiation using component-resolved diagnostics. *J Allergy Clin Immunol*. 2010;125:191-197.
39. Beyer K, Grabenhenrich L, Hartl M, et al. Predictive values of component-specific IgE for the outcome of peanut and hazelnut food challenges in children. *Allergy*. 2015;70:90-98.
40. Besharat PM, Bergstrom A, Bottai M, et al. Effect of parental migration background on childhood nutrition, physical activity, and body mass index. *J Obes*. 2014;2014:406529.

SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article.

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Table SI: Demographics of whole studied BAMSE population with available dust and *FLG* genotyping (n=1878)

	Number of cases	No. data points	Percentage of cases / no. of available data points
Peanut specific IgE $\geq$ 0.35kU/L at 4 years	103	1572	6.55%
Peanut specific IgE $\geq$ 0.35kU/L at 8 years	161	1876	8.58%
Ara h 1, 2 or 3 $\geq$ 0.35kU/L at 8 years vs. Ara h 1, 2 or 3 <0.35kU/L or peanut sIgE <0.35kU/L (excluding peanut sIgE $\geq$ 0.35kU/L with no components or missing data) at 8 years	75	1854	4.05%
History of infantile atopic dermatitis	313	1840	17.01%
Topical steroid use between age 1-2 years	534	1842	29.0%
Higher extent of Localization of eczema (>5 areas vs. <5 areas or no eczema)	49	1841	2.7%
Persistent eczema at age 8 years	321	1828	17.6%
At least one FLG mutation (R501X, 2282del or R2447x)	134	1878	7.14%
Allergic rhinitis (ISAAC definition) (age 4 years)	215	1828	11.76%
Allergic rhinitis (ISAAC definition) (age 8 years)	288	1865	15.44%
Egg sIgE $\geq$ 0.35IU/ml at 4 years of age	82	1575	5.21%
Reported allergic reaction to any food 8 years	349	1869	18.7%
Reported allergic reaction to peanut by 8 years	120	1869	6.4%
Reported allergic reaction to milk by 8 years	53	1869	2.8%
Reported allergic reaction to egg by 8 years	36	1869	1.9%
Reported allergic reaction to fish by 8 years	12	1869	0.6%
Reported allergic reaction to shellfish 8 years	17	1869	0.9%
Reported allergic reaction to wheat by 8 years	9	1869	0.5%
Reported allergic reaction to soya by 8 years	21	1869	1.1%
Reported allergic reaction to apple by 8 years	83	1869	4.4%
Reported allergic reaction to peach by 8 years	44	1869	2.4%
Reported allergic reaction to kiwi by 8 years	92	1869	4.9%

Reported allergic reaction to avocado 8 years	14	1869	0.7%
Reported allergic reaction to banana by 8 years	17	1869	0.9%
Reported allergic reaction to carrot by 8 years	53	1869	2.8%
Reported allergic reaction to tree nuts 8 years	105	1869	5.6%
Male sex	973	1871	52.00%
Caucasian ethnicity	1677	1842	91.04%
Biological siblings	1695	1870	90.64%
Parental atopy – (asthma/AR/eczema)	838	1858	45.10%
Parental atopy – (asthma/AR)	605	1856	32.60%
Maternal age (mean, SD)	30.9 (SD 4.49)	1871	N/A
Peanut protein in maternal bed-dust (µg/g): median [IQR]	4.07 [1.58-11.86]	1878	N/A
Exclusive breast-feeding duration (months): mean (SD)	5.19 (2.43)	1838	N/A
Total breast-feeding duration (months): mean (SD)	8.73 (3.31)	1825	N/A

Table S2: Univariate analysis of peanut specific IgE sensitization aged 4 and 8 years using GEE to account for repeated measures within individuals at 4 and 8 years and logistic regression for primary peanut sensitization at age 8 years in the whole BAMSE cohort (n=1878). Bold font used when p<0.05.

	GEE for peanut sIgE Univariate peanut sIgE sensitization at 4 and 8 years			Univariate primary peanut sensitization at age 8 years		
	OR	95% CI	P Value	OR	95% CI	P Value
EPE (µg/gram ln transformed)	0.95	0.86-1.06	0.39	1.01	0.86-1.19	.89
Male vs. female sex	1.14	0.83-1.56	0.43	1.41	0.88-2.26	.15
Full older siblings	1.08	0.63-1.85	0.77	0.86	0.40-1.81	0.68
≥1 <i>FLG</i> loss-of-function mutations	1.84	1.10-3.05	0.02	1.84	0.89-3.77	0.10
Questionnaire based history of infantile eczema	6.49	4.66-9.02	<.001	7.94	4.91-12.83	<.001
Localization of infantile eczema (continuous 1-10 areas/per unit)	1.48	1.37-1.59	<.001	1.53	1.40-1.68	<.001
Localization of infantile eczema (<5 areas vs. 5+ areas)	7.69	4.26-13.55	<.001	10.4	5.24-20.65	<.001
Topical steroids for itchy rash (1-2 years)	3.24	2.35-4.46	<.001	3.95	2.46-6.34	<.001
Persistent eczema at age 8 years	3.87	2.76-5.44	<.001	2.85	1.69-4.81	<.001
Egg sIgE sensitization at 4 years	13.58	8.55-21.58	<.001	11.04	6.13-19.90	<.001
Allergic reaction to milk by 8 years	5.68	3.12-10.34	<.001	4.24	1.84-9.77	0.001
Allergic reaction to egg by 8 years	26.81	13.98-51.4	<.001	16.67	7.81-35.59	<.001
Allergic reaction to fish by 8 years	17.25	5.41-54.96	<.001	9.18	2.39-35.32	0.001
Allergic reaction to shellfish 8 years	15.59	6.07-40.02	<.001	3.70	0.82-16.71	0.09
Allergic reaction to wheat by 8 years	9.55	2.53-36.02	0.001	8.06	1.60-40.59	0.01
Allergic reaction to soya by 8 years	48.75	17.61-134.97	<.001	41.95	16.56-106.24	<.001
Allergic reaction to apple by 8 years	12.54	8.16-19.27	<.001	5.93	3.15-11.13	<.001
Allergic reaction to peach by 8 years	14.37	8.15-25.34	<.001	6.49	2.88-14.61	<.001
Allergic reaction to kiwi by 8 years	5.95	3.80-9.31	<.001	4.25	2.20-8.20	<.001
Allergic reaction to avocado 8 years	12.27	4.61-32.66	<.001	4.38	0.95-20.13	0.06

Allergic reaction to banana 8 years	12.19	5.07-29.30	<.001	3.44	0.77-15.40	0.11
Allergic reaction to carrot by 8 years	17.84	10.6-30.05	<.001	7.83	3.83-16.03	<.001
Allergic reaction to tree nuts 8 years	21.93	14.45-33.28	<.001	10.29	12.08-34.05	<.001
Allergic rhinitis aged 4 years	2.82	1.92-4.13	<.001	2.16	1.22-3.83	0.008
Allergic rhinitis aged 8 years	8.05	5.78-11.22	<.001	5.87	3.66-9.42	<.001
Parental atopy (asthma and/or AR)	1.77	1.28-2.44	<.001	1.77	1.10-2.83	0.018
Parental atopy (asthma, AR, eczema)	1.44	1.05-1.98	0.02	1.50	0.95-2.39	0.09
Non-Caucasian ethnicity	1.58	0.97-2.57	0.07	1.09	0.49-2.43	0.79
Exclusively breastfed in months	0.98	0.94-1.06	0.92	0.97	0.88-1.06	0.49
Breastfed (exclusive/partial) in months	0.99	0.94-1.03	0.57	0.97	0.91-1.04	0.43
Maternal age at baseline (in years)	1.02	0.98-1.05	0.44	1.02	0.97-1.07	0.53

Table S3: Univariate analysis of factors associated with primary peanut sensitization (at age 8 years) versus non primary peanut sensitization (at age 8 years) in high risk children with preceding egg sensitization (at age 4 years) (n=134). Bold font used when p<0.05.

	Primary peanut sensitization vs. non-peanut sensitized egg sensitized controls		
	OR	95% CI	P Value
EPE (µg/gram In transformed)	1.33	1.01-1.74	<0.05
Male vs. female sex	1.90	0.95-3.80	0.07
Full older siblings	0.61	0.17-2.13	0.44
≥1 <i>FLG</i> loss-of-function mutations (excluding non-Caucasians)	1.09	0.33-3.56	0.89
History of infantile eczema	2.51	1.24-5.08	0.01
Localization of infantile eczema (continuous 1-10 areas/per unit)	1.15	1.01-1.32	0.04
Localization of infantile eczema (<5 areas vs. 5+ areas)	1.36	0.52-3.53	0.53
Application of topical steroids for itchy rash (1-2 years)	1.40	0.70-2.80	0.34
Persistent eczema at age 8 years	2.32	1.09-4.90	0.03
Allergic reaction to milk by 8 years	1.42	0.39-5.09	0.59
Allergic reaction to fish by 8 years	0.78	0.15-4.00	0.78
Allergic reaction to shellfish 8 years	0.30	0.06-1.58	0.16
Allergic reaction to wheat by 8 years	1.59	0.14-17.96	0.71
Allergic reaction to soya by 8 years	11.05	1.39-87.63	0.02
Allergic reaction to apple by 8 years	1.71	0.64-4.52	0.29
Allergic reaction to peach by 8 years	1.29	0.40-4.17	0.67
Allergic reaction to kiwi by 8 years	1.42	0.52-3.85	0.50
Allergic reaction to avocado 8 years	0.78	0.11-5.71	0.81
Allergic reaction to banana 8 years	0.38	0.07-2.13	0.27
Allergic reaction to carrot by 8 years	2.36	0.71-7.85	0.16
Allergic reaction to tree nuts 8 years	3.43	1.54-7.62	0.002
Allergic rhinitis aged 4 years (ISAAC)	0.87	0.38-1.96	0.73

Allergic rhinitis aged 8 years (ISAAC)	2.65	1.26-5.56	0.01
Parental atopy (asthma and /or hay-fever)	1.34	0.67-2.65	0.41
Non-Caucasian ethnicity	2.55	0.93-6.98	0.07
Exclusively breastfed in months	0.89	0.77-1.04	0.14
Breastfed (exclusive/partial) in months	0.99	0.89-1.09	0.76
Maternal age at baseline (in years)	1.06	0.99-1.14	0.11