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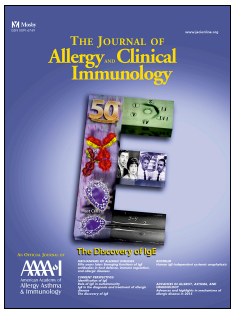

IgE Sensitization in Relation to Preschool Eczema and Filaggrin Mutation

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TITLE PAGE

Title

IgE sensitization in relation to preschool eczema and filaggrin mutation

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ABSTRACT

Background: Eczema (atopic dermatitis) is associated with an increased risk of having IgE antibodies. IgE sensitization may occur through an impaired skin barrier. Filaggrin (*FLG*) mutation is associated with eczema, and possibly also with IgE sensitization.

Objective: To explore the longitudinal relation between preschool eczema and/or *FLG* mutation and IgE sensitization in childhood.

Methods: A total of 3201 children from the BAMSE birth cohort recruited from the general population were included. Regular parental questionnaires identified children with eczema. Blood samples were collected at 4, 8, and 16 years for analysis of specific IgE. *FLG* mutation analysis was performed on 1890 of the children.

Results: Preschool eczema was associated with IgE sensitization to both food and aeroallergens up to age 16 (overall adjOR 2.30; 95%CI: 2.00-2.66). This association was even stronger among children with persistent preschool eczema. *FLG* mutation was associated with IgE sensitization to peanut at age 4 years (adjOR 1.88; 95% CI: 1.03-3.44), but not to other allergens up to age 16 years. *FLG* mutation and preschool eczema were not effect modifiers for the association between IgE sensitization and preschool eczema or *FLG* mutation, respectively. Sensitized children with preschool eczema were characterized by polysensitization, but no other specific IgE sensitization patterns were found.

Conclusions: Preschool eczema is associated with IgE sensitization, to both food and aeroallergens, up to 16 years of age. *FLG* mutation is associated with IgE sensitization to peanut, but not to other allergens. Sensitized children with preceding preschool eczema are more often polysensitized.

Key messages

- Preschool eczema is positively associated with IgE sensitization to food and aeroallergens up to age 16 years and this association is even stronger among children with persistent preschool eczema.
- *FLG* mutation irrespective of eczema is positively associated with IgE sensitization to peanut at age 4, but not to other food or aeroallergens up to age 16 years. This finding implies that *FLG* mutation without preschool eczema does not seem to play a major role in IgE sensitization.
- Sensitized children with a history of preschool eczema are more often polysensitized.

Capsule summary

Preschool eczema is associated with IgE sensitization to food and aeroallergens. *FLG* mutation is associated with IgE sensitization to peanut, but not other allergens. Preschool eczema is associated with polysensitization among sensitized children.

Key words

Allergens, atopic dermatitis, birth cohort, filaggrin, food allergy, IgE, peanut, population, sensitization, skin barrier

Abbreviations

adjOR: adjusted Odds Ratio

CI: Confidence intervals

77 *FLG*: Filaggrin gene

78 GEE: Generalized estimating equations

79 HDM: House dust mite

80 OR: Odds Ratio

81 PSE: Preschool eczema

82

83 **Word count:**

84 3,758

85 **Tables/Figures:**

86 6

87 **TEXT**

88 **INTRODUCTION**

89 Eczema (atopic dermatitis[1]) is an inflammatory skin disorder, commonly with onset during the first 2
90 years of life. Approximately 15-30% of children and 2-10% of adults in industrialized countries are
91 affected.[2] Eczema is associated with IgE-mediated food allergy,[3] and one third of children with eczema
92 will later develop asthma and/or rhinitis, the so-called atopic march.[4]
93 In 2003, Lack et al demonstrated that the use of skin preparations containing peanut oil was associated
94 with peanut allergy in children. They concluded that IgE sensitization to peanut protein may occur through
95 application of peanut oil to inflamed skin.[5] Defect skin barrier function in patients with eczema allows
96 penetration of allergens, irritants and bacteria.[6] Thus, sensitization to allergens may occur via the defective
97 skin barrier and has been suggested to initiate the atopic march.[7] Brough et al demonstrated that peanut
98 antigens in dust were associated with increased risk of IgE sensitization to peanut and peanut allergy in
99 atopic children. The risk was higher in children with a history of eczema and severe eczema.[8]
100 Loss-of-function mutation in the filaggrin gene (*FLG*) increases the risk of eczema[9] and also of IgE
101 sensitization according to a systematic review and meta-analysis.[10] However, recent studies evaluating the
102 association between *FLG* mutation and IgE sensitization, and including eczema as a covariate, present
103 conflicting results.[11-15] The *FLG* gene encodes an epidermal protein which plays a key role for skin barrier
104 function.[16] *FLG* mutations, in the absence of eczema, have been shown to be associated with peanut
105 allergy.[17] In addition, early-life exposure to environmental peanut is reported to be related to increased
106 risk of peanut allergy in children who carry a *FLG* mutation.[18] These findings support the idea of IgE
107 sensitization through an impaired skin barrier. However, children without visible skin lesions and without
108 any *FLG* mutation also develop IgE sensitization, and a significant proportion of children with eczema
109 never become IgE-sensitized.[6]
110 It is well-known that children with eczema have increased prevalence of IgE antibodies to common
111 allergens, compared with children without eczema.[19, 20] However, potential differences in patterns of IgE

sensitization between sensitized children with and without preschool eczema (PSE) and/or *FLG* mutation have not been explored.

We hypothesized that PSE and/or *FLG* mutation are associated with IgE sensitization in childhood, possibly through skin barrier dysfunction, and performed a secondary analysis of the BAMSE database. In addition, we explored if sensitized children with PSE and/or *FLG* mutation present different patterns of IgE sensitization than sensitized children without PSE or *FLG* mutation.

METHODS

Study design and setting

The BAMSE project, which has been described in detail elsewhere,[21] is a population-based cohort including 4089 children followed from birth up to age 16. Detailed data on residential characteristics, environmental factors and parental allergy were obtained when the children were around 2 months old from a baseline parental questionnaire. When the children were 1, 2, and 4 years, parents completed questionnaires on manifestations of eczema, asthma and rhinitis during the preceding 12 months as well as on doctor's diagnosis of disease since last follow-up. At the 16-year follow-up, 3181 (78%) subjects still remained in the cohort. The design of the current study was done post hoc to data collection, but the hypotheses being tested were derived prior to the interrogation of the dataset.

Blood analyses

Blood was collected for analysis of serum IgE antibodies to a mix of common airborne and food allergens at 4, 8, and 16 years. Airborne allergens were analyzed with Phadiatop® (cat, dog, horse, birch, timothy, mugwort, house dust mite (*Dermatophagoides pteronyssinus at 4 and 8, Dermatophagoides pteronyssinus and/or Dermatophagoides farinae* at 16) and mold (*Cladosporium herbarum*)), and food allergens with fx5® (cow's milk, hen's egg, cod, soy, peanut, and wheat), using the ImmunoCAP System (Thermo Fisher Scientific, Uppsala, Sweden). The results were expressed as positive (≥0.35 kU_A/L) or negative (<0.35 kU_A/L). To be classified as sensitized at each respective age, a child was required to be positive for Phadiatop and/or fx5. Sera with Phadiatop or fx5 results of ≥0.35 kU_A/L were subsequently analyzed for IgE antibodies to the airborne and food allergens listed above. Sensitization to a specific allergen at each respective age was defined as IgE ≥0.35 kU_A/L to that allergen. All samples were analyzed in accordance with the instructions of the manufacturer at the Department of Clinical Immunology, Karolinska University Hospital Solna, Stockholm, Sweden. In addition, genotyping for the *FLG* mutations common in Scandinavia was done among children who provided blood at 8 years of age.[22] The genotyping was performed using TaqMan allelic discrimination for R501X and R2447X on the ABI Prism 7500 detection system in accordance with the manufacturer's protocol (Applied Biosystems, CA, USA) and MALDI-

144 TOF (matrix-assisted laser desorption/ionisation-time of flight; Sequenom GmbH, Hamburg, Germany)
145 for 2282del4. Primer sequences, amplification conditions, and allele sizes are available on request.
146 Children with a mutation in any of the positions 2282del4, R501X or R2447X were classified as having a
147 *FLG* mutation.

148 **Study population**

149 Children with data on preschool eczema (n=3700) who provided blood at least once (n=3316) constituted
150 the study population (n=3201, 78.3% of the original cohort). *FLG* mutation-analysis was available for a
151 subset of 59.0% of children in the study population. When analyzing the pattern of IgE sensitization,
152 children who had complete data on specific IgE, and who were IgE-positive for aeroallergens and/or food
153 allergens, were included (Fig 1).

154 **Definitions**

155 *Preschool eczema (PSE)*; parental reports of dry skin and itchy rash for 2 weeks or more with specific
156 locations of rash (face or arm/leg extension surfaces or arm/leg flexures or wrist/ankles or neck) in the last
157 12 months and/or doctor's diagnosis of eczema since the last follow-up at one or more of the follow-ups at
158 age 1, 2, and 4 years.[23]

159 *Persistent PSE*; eczema at age 1, 2, and 4.

160 *Severe PSE*; eczema in combination with sleep disturbance due to itch at age 1, 2, and/or 4 years.

161 *Monosensitization*; IgE ≥0.35 kU_A/L to a single allergen, or positive for Phadiatop and/or fx5 without
162 reaching ≥0.35 kU_A/L to any of the allergens included in the mixes.

163 *Oligosensitization*; IgE ≥0.35 kU_A/L to 2-3 allergens.

164 *Polysensitization;* IgE ≥0.35 kU_A/L to ≥4 allergens.

165 **Statistics**

166 Background characteristics were expressed as percentage of the total number of individuals observed and
167 95% confidence intervals (95% CI) adjusted for finite population sampling.[24] To assess the association
168 between IgE sensitization (any, food and aeroallergens) at 4, 8, and 16 years and preschool eczema, and/or

FLG mutations, we used a logistic regression model using generalized estimating equations (GEEs) with an unstructured correlation matrix to account for the correlation between repeated outcomes. Unstructured correlation was used due to the longitudinal design and the unequal spacing to the follow-up point in time. GEE analyses were performed on both prevalent and incident cases at 8 and 16 years. Potential effect modifiers for the association between PSE and *FLG* mutation, respectively, and IgE sensitization, were investigated using logistic regression for each covariate separately (sex, heredity, breastfeeding, parental smoking during infancy, mother's age, socio-economy, *FLG* mutation and, in *FLG* mutation-analysis, PSE). To investigate if associations between PSE and sensitization outcomes were different at different ages, we added an interaction term to the model. A separate model was used for each outcome: any sensitization, sensitized to food allergens and sensitized to aeroallergens. The same procedure was repeated for the effect of *FLG* mutation. We decided a priori to include sex and heredity in the models and other potential confounders (listed above) were included if the univariate analysis changed the Odds Ratio (OR) more than 10%. Logistic regression was also used when calculating ORs for the association between PSE and mono-, oligo-, and polysensitization (children without IgE sensitization were the reference category). In the investigator-driven pattern analysis, χ2-tests were used for dichotomous variables. P values <.05 were considered statistically significant. The analyses above were not adjusted for multiple hypothesis testing. For the data-driven pattern analysis (cluster analysis) we included all allergens as dichotomous variables (positive or negative). Due to our large data set we used the k-means method with Euclidean distance between the observations, since this gave the most stable clusters compared with other methods tested (Jaccard and matching). This analysis was performed separately at age 4, 8 and 16 years. We tested 2-, 3-, 4-, and 5-cluster models, and selected the clusters that maximized the Calinski-Harabasz pseudo-F.[25] Cluster analysis was initially performed separately for sensitized children with and without PSE at each age and thereafter including all sensitized children at each age. All statistical calculations were performed with Stata Statistical Software (release 12.1; StataCorp, College Station, TX, USA).

Ethics

This study was approved by the Regional Ethical Review Board at Karolinska Institutet, Stockholm.

Informed consent was provided by teenagers and guardians.

RESULTS

Participants and descriptive data

There were no differences in background characteristics between the study participants (n=3201) and the original cohort, with exception of socio-economy and heredity (see Table E1 in the Online Repository). In the study population, 1107 (34.6%) had PSE and, of these, 381 (34.4%) had severe PSE, while 214 (19.3%) persistent PSE. Among the 1890 children analyzed for *FLG* mutation, 137 (7.3%) had any *FLG* mutation and, of these, 48.9% (n=67) had PSE. *FLG* mutation was more common among children with PSE compared with children without PSE (10.1% vs. 5.7%, P<0.001).

Logistic regression analysis: IgE sensitization in relation to PSE

A history of PSE was positively associated with any IgE sensitization (overall adjusted Odds Ratio (adjOR) 2.39; 95% CI: 2.00-2.63) from 4 to 16 years. This positive association was found for both food allergens (adjOR 2.05; 95% CI: 1.74-2.42) and aeroallergens (adjOR 2.51; 95% CI: 2.18-2.92), up to age 16 years when analyzed separately. The highest OR associated with PSE was for aeroallergens at age 4 years (Fig 2A). The point estimates were higher for children with severe PSE and significantly higher for children with persistent PSE for both food and aeroallergens, with the strongest association for sensitization to airborne allergens at 4 years (adjOR 9.28; 95% CI: 6.64-12.97) (Table I).We also analyzed the association between PSE and IgE sensitization to separate allergens at 4, 8, and 16 years and found that PSE was significantly associated with IgE sensitization to almost all of the 14 included allergens. (see Table E2 in the Online Repository). PSE was also associated with incident IgE sensitization at 8 years (adjOR 1.44; 95% CI: 1.08-1.91) and 16 years (adjOR 1.48; 95% CI: 1.08-2.03). *FLG* mutation was not a significant effect modifier to the effect of PSE on IgE sensitization, and adjustment for *FLG* mutation did not change the ORs (data not shown). Both preschool asthma and preschool rhinitis were significant effect modifiers of the effect of PSE on IgE sensitization. Stratification by preschool asthma and rhinitis revealed that the OR for any IgE sensitization, food allergens and aeroallergens was more pronounced among children with preschool asthma or rhinitis (data not shown). The overall OR for the association

between PSE and any sensitization up to 16 years among children without asthma or rhinitis before age 4 years was still significant (adjOR 1.85; 95% CI: 1.57-2.19).

Logistic regression analysis: IgE sensitization in relation to *FLG* mutation

FLG mutation was not associated with IgE sensitization at age 4, 8 or 16 years (Fig 2B). However, when we evaluated all allergens separately, *FLG* mutation was associated with IgE sensitization to peanut at 4 years (adjOR 1.88, 95% CI: 1.03-3.44) and to house dust mite (HDM) at 16 years (adjOR 0.47; 95% CI: 0.23-0.96), but not to other allergens (see Table E3 in the Online Repository). PSE did not modify the effect of *FLG* mutation on IgE sensitization, and stratification by PSE and heredity resulted in minor differences.

IgE sensitization in the whole study population

The proportions of any IgE sensitization increased over time from 24.3% (622 of 2560) at 4 years, 35.0% (832 of 2378) at 8 years to 46.1% (1135 of 2464) at 16 years. Children with PSE had an increased risk for mono-, oligo-, and polysensitization at all ages compared with children without PSE. The risk was most pronounced at 4 years; mono (adjOR 1.79; 95% CI: 1.40-2.30), oligo (adjOR 2.73; 95% CI: 2.01-3.72), poly (adjOR 7.91; 95% CI: 5.18-12.08).

Patterns of IgE sensitization among sensitized children

In the subpopulations (children with any IgE sensitization at 4, 8 or 16 years), monosensitization was most common among children without PSE, while polysensitization was most common among children with PSE (Fig 3). Among sensitized children, children with PSE had a higher prevalence of IgE sensitization to most allergens than children without PSE. However, sensitization to HDM, milk and fish at 4, 8, and 16 years did not differ between children with and without PSE (Fig 4).

There were no differences in mono-, oligo-, and polysensitization when comparing sensitized children with and without *FLG* mutation (see Table E4 in the Online Repository). However, when we explored the prevalence of single allergens we found that IgE antibodies to peanut were significantly more common among children with *FLG* mutation; at 4 years (43.3% vs. 22.3%, P=0.010) and at 8 years (36.0% vs. 21.8%, P=0.022), but not at 16 years (16.4% vs. 17.1%, P=0.89). Children with *FLG* mutation in

combination with PSE had the highest prevalence of IgE sensitization to peanut at 4 years and 8 years (Table II). There were no other significant differences in IgE sensitization to single allergens when comparing those with and without *FLG* mutation (see Table E4 in the Online Repository).

Cluster analysis of IgE sensitization

Finally, we used a data-driven approach and performed a cluster analysis on all 14 allergens stratified by PSE to evaluate if there were any specific patterns of sensitization among children with and without a history of PSE. We found that a 2-cluster model was the most stable model at all ages for sensitized children with PSE. For sensitized children without PSE no stable cluster model was found and we were therefore unable to compare sensitization patterns between sensitized children with and without a history of PSE. Instead, we performed cluster analysis including all children with any IgE sensitization at the ages 4, 8, and 16 years. The 2-cluster model was most stable and gave the highest Calinski-Harabasz pseudo-F at all ages.[25] The first cluster was characterized by mono- and oligosensitization, and the second cluster was characterized by polysensitization. PSE was significantly more common in the latter. This difference decreased with age but was still significant at 16 years (54.6% vs. 38.8%, P<0.001). The cluster analysis did not provide any additional patterns of IgE sensitization associated with PSE. *FLG* mutation was equally common in both clusters (at 4 years: 7.8% vs 8.3%, P=0.89; at 8 years: 6.7% vs. 9.6%, P=0.20; at 16 years: 8.4% vs. 6.1%, P=0.22) (see Table E5 in the Online Repository).

DISCUSSION

Main findings

In this population based birth cohort, we explored IgE sensitization in relation to both preschool eczema (PSE) and *FLG* mutation and found that PSE was positively associated with IgE sensitization to both food and aeroallergens at age 4, 8, and 16 years. This association was even stronger among children with a history of persistent PSE. *FLG* mutation was positively associated with IgE sensitization to peanut at 4 years, but not with IgE sensitization to other common food or aeroallergens. When exploring patterns of IgE sensitization to 14 single allergens among sensitized children, we found that the group with a history of PSE was characterized by polysensitization and higher prevalence of IgE antibodies to most food and aeroallergens at age 4, 8 and 16 years. We also found that sensitized children with *FLG* mutation had a higher prevalence of IgE sensitization to peanut at 4 and 8 years. Other sensitization patterns did not differ between sensitized children with and without *FLG* mutation.

Strengths and limitations

This study has several strengths, especially the large number of participants recruited from the general population with follow-up from birth to 16 years. The eczema criteria in the questionnaire have been validated with a sensitivity and specificity of 92% and 100%, respectively.[26] Thus, there is a decreased risk of misclassification of our collected PSE data as well as for several other prospectively collected covariates. This allows for adjustment for potential confounders, including parental allergy. In addition, IgE sensitization was measured at three time points, including measurement of a large number of food and airborne allergens. Another advantage was that we used both an investigator-driven and a data-driven (cluster analysis) approach when analyzing patterns of sensitization. One limitation is that we did not have data on IgE sensitization before age 4 years. Thus, we could not explore if IgE sensitization preceded symptoms of eczema or vice versa. Another limitation is that *FLG* mutation data was not available in all participants, but on the other hand, few longitudinal studies have analyzed *FLG* mutation in nearly 1900 patients.

Results in the context of the existing literature

We found a positive association between PSE and any IgE sensitization up to age 16 years. In the Isle of Wight cohort they analyzed IgE sensitization to food and aeroallergens in relation to eczema up to age 4 years and found an association comparable with our result at 4 years.[20] In line with previous research, we found a clear association between IgE sensitization to food allergens and PSE.[3, 27] Flohr et al showed that the association between eczema and food sensitization was stronger with disease severity.[13] We made a similar finding and in addition we found that children with persistent PSE had even higher point estimates for food sensitization. Few studies have investigated the association between PSE and sensitization to aeroallergens later in life. We were surprised to find even stronger associations between PSE and airborne allergens than between PSE and food allergens. Notably, these associations were strongest at 4 years, even though sensitization to airborne allergens is more prevalent later in life.

We hypothesized that *FLG* mutation is associated with IgE sensitization and in this large material we found that *FLG* mutation, irrespective of eczema, was associated with IgE sensitization to peanut at 4 years. These findings are in line with previous studies having shown that *FLG* mutation is associated with both peanut sensitization and to peanut allergy.[17, 18] However, we did not find any other significant associations between *FLG* mutation and IgE sensitization to other common food allergens. Several studies have investigated the association between *FLG* mutations and IgE sensitization to foods with conflicting results. In contrast to our findings, Tan et al found that *FLG* mutation was associated with IgE sensitization to food, also after adjustment for eczema.[11] Other studies have also found an association between *FLG* mutation and IgE sensitization to foods, but this association was not evident in the absence of prior eczema.[12, 14] Later studies have not found an association between IgE sensitization to food allergens and *FLG* mutation.[13, 15]

We also found that *FLG* mutation was associated with IgE sensitization to HDM at age 16 years (see Table E4 in the Online Repository). Very few studies have analyzed the association between *FLG* mutation and IgE sensitization to aeroallergens and there is so far no support of our findings in the literature[12, 15]. Thus it is possible that the association between *FLG* mutation and HDM occurred by chance due to multiple testing. We cannot rule out that this was also the case for the association between *FLG*

316 mutation and peanut sensitization, but in view of previous publications we consider this less likely.[18]
317 Environmental peanut exposure in dust has been shown to be related to peanut sensitization possibly
318 through the skin.[18, 28] However, other food and aeroallergens including HDM are also found in house
319 dust.[29, 30] Additional studies are needed to understand why *FLG* mutation is associated with IgE
320 sensitization to certain allergens but not to others.
321 We have, for the first time, compared patterns of IgE sensitization among sensitized children in relation to
322 PSE and *FLG* mutations. The finding that sensitized children with a history of PSE displayed a higher
323 degree of polysensitization at all ages compared with sensitized children with no history of PSE is new
324 and adds to recent publications concerning IgE polysensitization.[31-33] Among sensitized children, children
325 with PSE had higher prevalence of IgE sensitization to most common food and aeroallergens. In contrast,
326 *FLG* mutation among sensitized children was not associated with polysensitization and the only pattern
327 that we found among these children was that sensitization to peanut was more common at age 4 and 8
328 years.
329 Our results support the idea that eczema increases the risk of IgE sensitization, possibly through skin
330 barrier dysfunction. We believe that some findings in particular point towards a causal relationship
331 between skin barrier dysfunction and IgE sensitization: i) that the strongest associations between eczema
332 and IgE sensitization to food and airborne allergens was found at 4 years, even though sensitization to
333 airborne allergens is more prevalent later in life, ii) that the associations between eczema and IgE
334 sensitization were significantly stronger among children with persistent eczema, representing a group with
335 longer exposure regarding skin barrier dysfunction, and iii) that sensitized children with a history of
336 eczema more often displayed polysensitization compared with sensitized children without a history of
337 PSE.
338 However, the complex etiology of atopic diseases makes causal inference difficult and our findings might
339 be explained by other factors. By including only sensitized children when analyzing patterns of
340 sensitization in relation to PSE or *FLG* mutations, we ensured that the comparison was made among
341 individuals with a similar phenotype or genotype, disposed to produce specific IgE. The material also

enabled us to adjust for parental allergy. However, eczema and skin barrier dysfunction alone cannot explain the development of IgE sensitization and we have previously shown that almost 50% of children who display any IgE sensitization up to 16 years have no history of eczema.[34] Thus, an alternative explanation to our findings might be that there are various genetic phenotypes predisposing to eczema and sensitization, regardless of skin barrier dysfunction.

Conclusions

We show that eczema in the first four years of life is strongly associated with IgE sensitization both to food and aeroallergens throughout childhood. For children who have had persistent eczema this association is even stronger. Our study confirms previously described associations between *FLG* mutation and IgE sensitization to peanut, but not to other allergens. This finding implies that *FLG* mutation without preschool eczema does not seem to play a major role in IgE sensitization. Our results highlight the importance of including data on eczema in the first years of life in future studies aiming to explore *FLG* mutations in relation to IgE sensitization.

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Figure legends

FIG 1

Flow chart of the study showing children included in different analyzes of IgE sensitization in relation to preschool eczema□ (PSE) and filaggrin (*FLG*) mutation.

Footnotes:

□PSE was defined as eczema between 0 and 4 years of age based on questionnaire data

*Generalized Estimating Equations

FIG 2A

Association between preschool eczema (PSE) and IgE sensitization at 4, 8, and 16 years of age.

Footnotes:

Children with data on PSE who provided blood at least once were included in the analysis (n=3201)

Logistic regression model using generalized estimating equations (GEEs), adjusted for sex and heredity

Effect modification was tested for sex, heredity, breastfeeding, parental smoking during infancy, mother's age, socio-economy and filaggrin mutation

FIG 2B

Association between filaggrin (*FLG)* mutation and IgE sensitization at 4, 8, and 16 years of age.

Footnotes:

Children from the BAMSE cohort with data on *FLG* mutation and preschool eczema (PSE) were included in the analysis (n=1890)

Logistic regression model using generalized estimating equations (GEEs), adjusted for PSE, sex, heredity

Effect modification was tested for sex, heredity, breastfeeding, parental smoking during infancy, mother's age, socio-economy and PSE

FIG 3

Proportions of children with mono-, oligo-, and polysensitization among IgE-sensitized children with and without preschool eczema (PSE) at 4, 8, and 16 years in the BAMSE cohort.

FIG 4

Pattern of IgE sensitization among sensitized children with and without a history of preschool eczema (PSE).

Footnotes:

Children in the BAMSE cohort with any IgE sensitization and complete data on specific IgE at the respective age were included

Significant differences are marked with *

House dust mite (HDM)

TABLE I IgE sensitization at age 4, 8, and 16 years in relation to preschool eczema (PSE), severe PSE, persistent PSE and PSE in combination with *FLG* mutation.

	PSE (n=1107)		Severe PSE[b] (n=381)		Persistent PSE[c] (n=214)		PSE and *FLG* mutation[d] (n=67)	
	adjOR[e]	95% CI	adjOR[e]	95% CI	adjOR[e]	95% CI	adjOR[e]	95% CI
Any sensitization[e]								
4	2.55	2.12-3.06	3.87	3.01-4.98	6.07	4.40-8.37	2.74	1.53-4.88
8	2.20	1.85-2.60	3.08	2.41-3.94	5.04	3.62-7.02	2.06	1.24-3.43
16	2.21	1.87-2.61	3.12	2.39-4.06	4.99	3.41-7.29	2.52	1.44-4.42
Foods[e]								
4	2.10	1.70-2.59	2.96	2.23-3.91	4.34	3.11-6.06	2.30	1.20-4.40
8	2.04	1.67-2.49	3.05	2.33-3.98	4.77	3.45-6.60	2.80	1.64-4.79
16	2.01	1.59-2.54	3.00	2.20-4.08	5.30	3.71-7.56	2.56	1.32-4.95
Airborne[e]								
4	3.37	2.71-4.19	5.54	4.19-7.34	9.28	6.64-12.97	3.52	1.84-6.72
8	2.49	2.08-2.99	3.47	2.69-4.48	5.90	4.27-8.16	2.02	1.18-3.46
16	2.26	1.91-2.68	3.33	2.56-4.33	5.00	3.45-7.25	2.74	1.56-4.82

[a]Children with the result ≥0.35 kU_A/L were classified as having IgE sensitization

[b]Children with sleep disturbance in combination with eczema at 1, 2, and/or 4 years of age. Children with PSE without sleep disturbance and missing data at one or two ages were excluded, children without PSE (n=2094) were the reference

[c]Children with eczema at 1, 2, and 4 years of age. Children without PSE (n=2094) were the reference

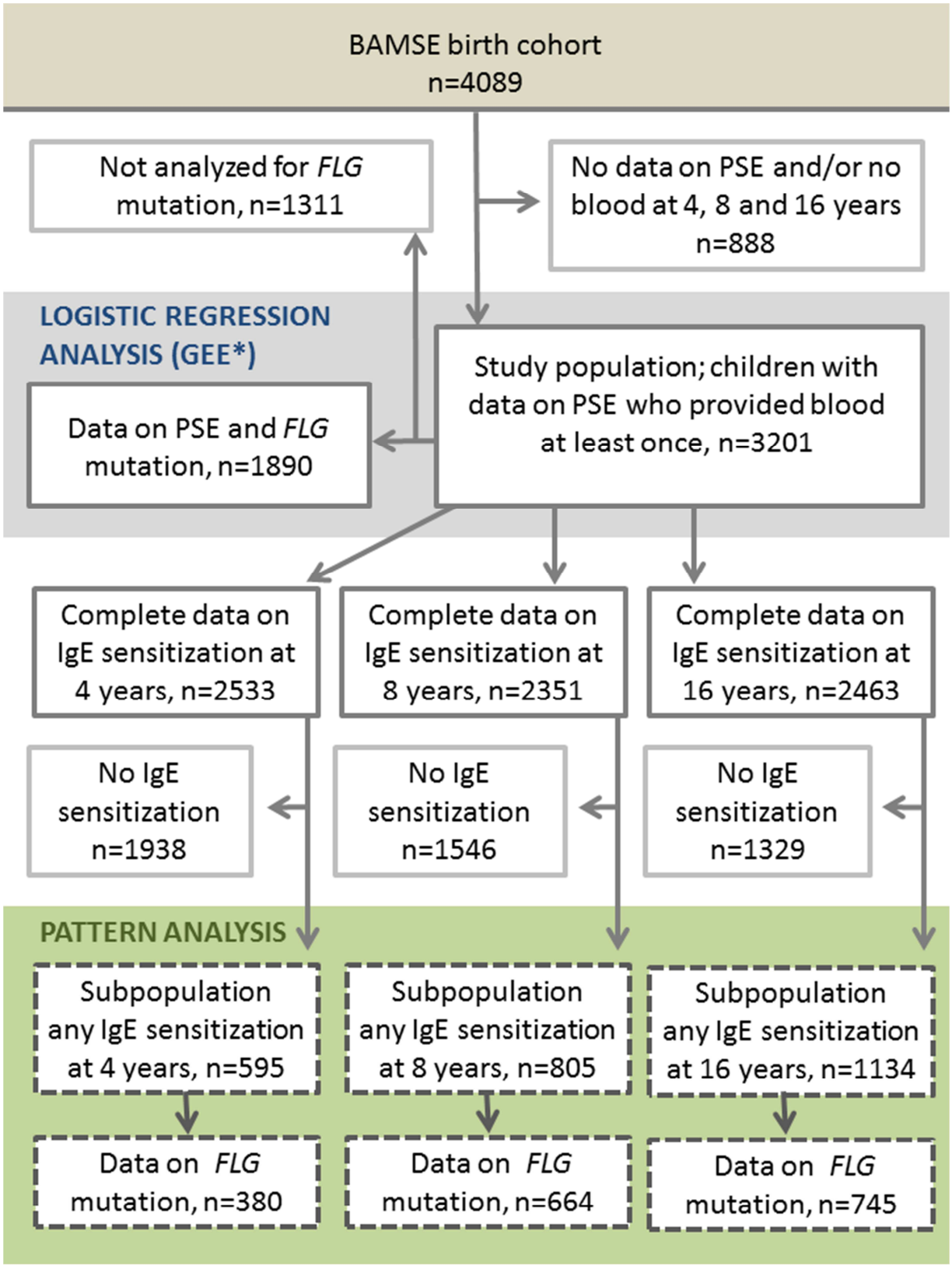
[d]Children without PSE and *FLG* mutation (n=1157) were the reference

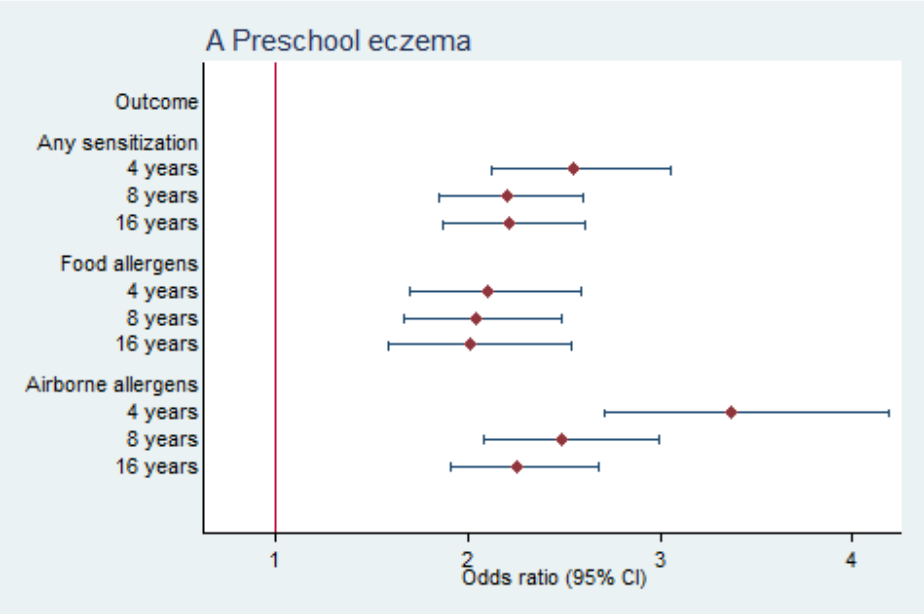
[e]Logistic regression model using generalized estimating equations (GEEs), adjusted for sex and heredity. Effect modification was tested for sex, heredity, breastfeeding, parental smoking during infancy, mother's age, socio-economy and filaggrin mutation

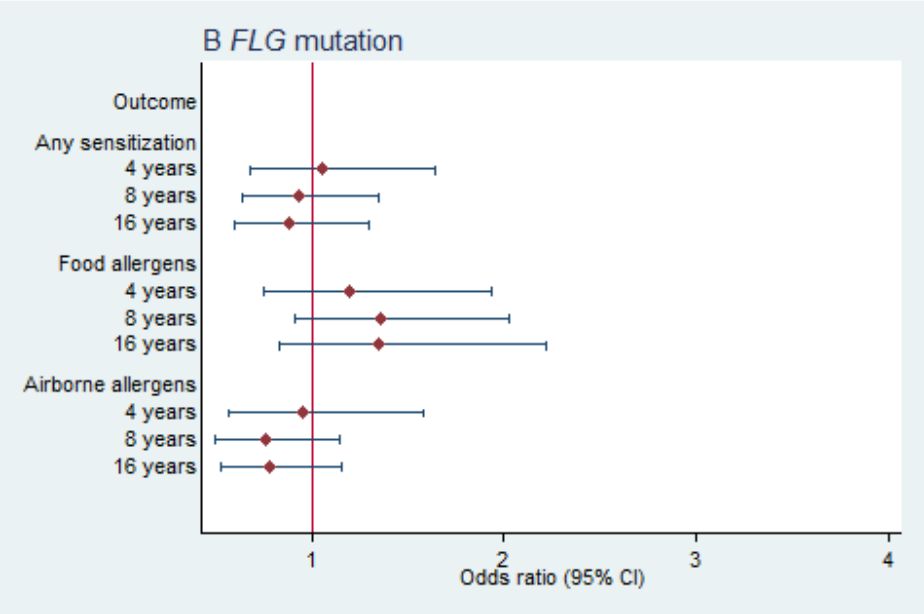
TABLE II IgE sensitization to peanut at 4, 8, and 16 years among children with preschool eczema (PSE+) and/or filaggrin mutation (*FLG*+).

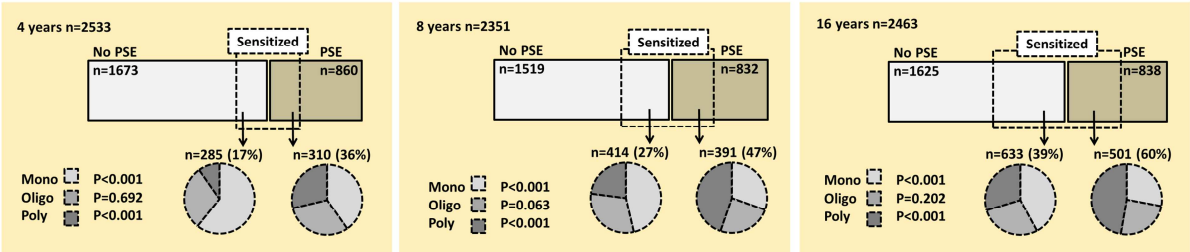
	4 years			**8 years**			**16 years**		
	IgE sensitization to peanut			IgE sensitization to peanut			IgE sensitization to peanut		
	N	%	95% CI	N	%	95% CI	N	%	95% CI
PSE-/*FLG*- (reference)[a]	25/161	15.5	10.3-22.1	48/324	14.8	11.1-19.2	47/387	12.1	9.1-15.8
PSE+/*FLG*-	53/189	28.0	21.8-35.0	**86/290**	**29.7**	**24.5-35.3**	**71/303**	**23.4**	**18.8-28.6**
PSE-/*FLG*+	4/11	36.4	10.9-69.2	6/20	30.0	11.9-54.3	3/19	15.8	3.4-39.6
PSE+/*FLG*+	**9/19**	**47.4**	**24.4-71.1**	**12/30**	**40.0**	**22.7-59.4**	6/36	16.7	6.4-32.8

[a]Children without preschool eczema (PSE-) and *FLG* mutation (*FLG*-) were the reference
Significant differences in bold


BAMSE birth cohort
n=4089
Not analyzed for FLG mutation, n=1311
No data on PSE and/or no blood at 4, 8 and 16 years n=888
LOGISTIC REGRESSION ANALYSIS (GEE*)
Study population; children with data on PSE who provided blood at least once, n=3201
Data on PSE and FLG mutation, n=1890
Complete data on IgE sensitization at 4 years, n=2533
Complete data on IgE sensitization at 8 years, n=2351
Complete data on IgE sensitization at 16 years, n=2463
No IgE sensitization n=1938
No IgE sensitization n=1546
No IgE sensitization n=1329
PATTERN ANALYSIS
Subpopulation any IgE sensitization at 4 years, n=595
Subpopulation any IgE sensitization at 8 years, n=805
Subpopulation any IgE sensitization at 16 years, n=1134
Data on FLG mutation, n=380
Data on FLG mutation, n=664
Data on FLG mutation, n=745







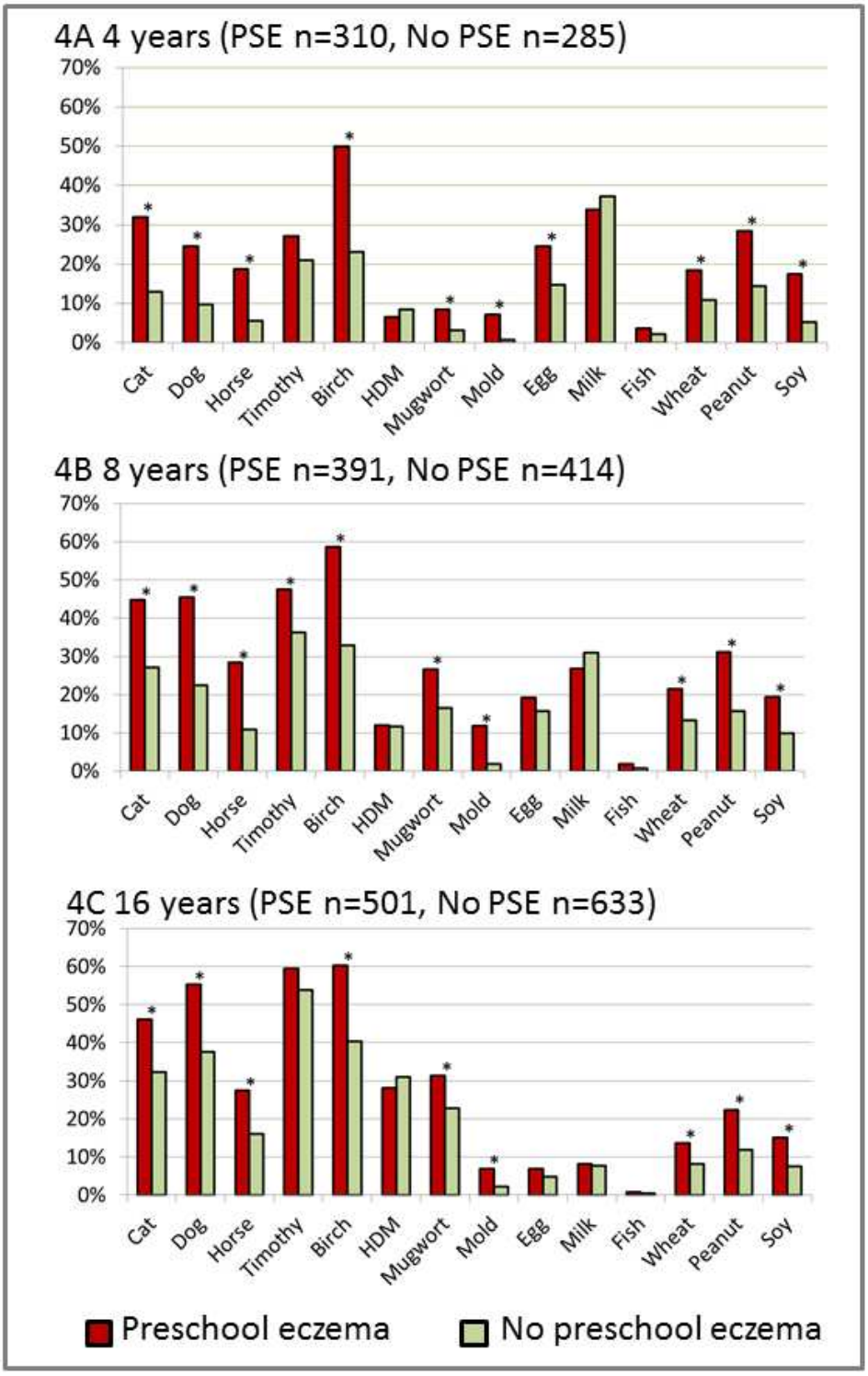

4A 4 years (PSE n=310, No PSE n=285)
70%
60%
50%
40%
30%
20%
10%
0%
Cat
Dog
Horse
Timothy
Birch
HDM
Mugwort
Mold
Egg
Milk
Fish
Wheat
Peanut
Soy
4B 8 years (PSE n=391, No PSE n=414)
70%
60%
50%
40%
30%
20%
10%
0%
Cat
Dog
Horse
Timothy
Birch
HDM
Mugwort
Mold
Egg
Milk
Fish
Wheat
Peanut
Soy
4C 16 years (PSE n=501, No PSE n=633)
70%
60%
50%
40%
30%
20%
10%
0%
Cat
Dog
Horse
Timothy
Birch
HDM
Mugwort
Mold
Egg
Milk
Fish
Wheat
Peanut
Soy
Preschool eczema
No preschool eczema

TABLE E1

Background characteristics for children in the original cohort and the study population

	Original cohort		Study population		
	N=4089	%	N=3201	%	95% CI
Male	2065/4089	50.5	1617/3201	50.0	49.7-51.3
Low socio-economy[a]	695/4072	17.1	515/3189	**16.2**	**15.6-16.8**
Exclusive breastfeeding >4 months	3116/3919	79.5	2549/3181	80.1	79.5-80.7
Heredity[b]	1746/4045	43.2	1415/3175	**44.6**	**43.8-45.4**
Parental smoking[c]	855/4067	21.0	653/2532	20.5	19.7-21.4
Any parent born outside Scandinavia[d]	543/3398	16.0	468/2990	15.7	15.2-16.1
Young mother[e]	319/4088	7.8	244/3201	7.6	7.2-8.1

Confidence intervals (CI) adjusted for finite population sampling. Statistically significant differences in bold

[a]Both parents blue-collar workers

[b]Mother and/or father with doctor's diagnosis of asthma and/or hay fever and/or eczema (contact allergy among parents is excluded) at baseline

[c]Any of the parents smoked at least one cigarette per day at birth

[d]Parent not born in Sweden, Denmark, Norway or Finland

[e]Mother's age ≤25 years at birth

TABLE E2

Associations between preschool eczema (n=1107) and IgE sensitization to common food and aeroallergens at 4, 8, and 16 years in the study population (n=3201).

	4 years		8 years		16 years		Overall up to 16 years	
	adjOR[a]	95% CI	adjOR[a]	95% CI	adjOR[a]	95% CI	adjOR[a]	95% CI
Cat	**4.85**	**3.38-6.96**	**2.95**	**2.32-3.76**	**2.45**	**2.00-3.01**	**2.77**	**2.28-3.35**
Dog	**5.07**	**3.35-7.66**	**3.89**	**3.00-5.03**	**2.86**	**2.35-3.49**	**3.16**	**2.62-3.82**
Horse	**7.79**	**4.53-13.37**	**4.67**	**3.32-6.58**	**2.87**	**2.19-3.76**	**3.56**	**2.77-4.58**
Timothy	**2.59**	**1.86-3.61**	**2.49**	**1.98-3.12**	**1.90**	**1.58-2.28**	**2.08**	**1.75-2.47**
Birch	**4.82**	**3.61-6.42**	**3.56**	**2.85-4.43**	**2.82**	**2.34-3.41**	**3.18**	**2.66-3.80**
HDM[b]	1.56	0.87-2.81	**1.67**	**1.12-2.49**	**1.42**	**1.12-1.80**	**1.46**	**1.16-1.83**
Mugwort	**4.92**	**2.45-9.89**	**2.75**	**2.01-3.77**	**2.33**	**1.83-2.97**	**2.49**	**1.98-3.14**
Mold	**19.56**	**4.97-76.99**	**8.34**	**4.23-16.42**	**6.36**	**3.25-12.46**	**8.57**	**4.91-14.97**
Egg	**3.76**	**2.57-5.50**	**1.95**	**1.39-2.75**	**2.01**	**1.22-3.31**	**2.51**	**1.90-3.30**
Milk	**2.02**	**1.53-2.67**	**1.52**	**1.15-1.99**	**1.63**	**1.06-2.50**	**1.72**	**1.39-2.14**
Fish	**3.33**	**1.24-8.93**	**3.90**	**1.02-14.92**	2.37	0.49-11.50	**3.30**	**1.40-7-75**
Wheat	**3.65**	**2.40-5.55**	**2.91**	**2.06-4.10**	**2.55**	**1.75-3.71**	**2.96**	**2.25-3.89**
Peanut	**4.24**	**2.97-6.04**	**3.86**	**2.86-5.20**	**3.05**	**2.86-5.20**	**3.56**	**2.73-4.63**
Soy	**6.95**	**4.08-11.86**	**3.73**	**2.55-5.45**	**3.43**	**2.38-4.95**	**4.16**	**3.02-5.74**

[a]Logistic regression model using generalized estimating equations (GEEs), adjusted for sex and heredity. Effect modification was tested for sex, heredity, breastfeeding, parental smoking during infancy, mothers age, socio-economy and filaggrin mutation

Children without PSE (n=2094) were the reference

[b]House dust mite (HDM)

Significant differences in bold

TABLE E3

Associations between filaggrin (*FLG*) mutation (n=137) and IgE sensitization to common food and aeroallergens at 4, 8, and 16 years and in the study population (n=1890).

	4 years		8 years		16 years		Overall up to 16 years	
	adjOR[a]	95% CI	adjOR[a]	95% CI	adjOR[a]	95% CI	adjOR[a]	95% CI
Cat	0.66	0.28-1.57	0.73	0.42-1.57	0.88	0.55-1.42	0.82	0.52-1.29
Dog	1.05	0.47-2.36	1.18	0.72-1.93	0.68	0.42-1.09	0.81	0.52-1.25
Horse	1.21	0.50-2.93	1.28	0.70-2.34	1.05	0.59-1.85	1.12	0.68-1.87
Timothy	1.12	0.54-2.32	0.76	0.45-1.27	0.92	0.60-1.40	0.89	0.60-1.32
Birch	1.28	0.73-2.26	0.82	0.50-1.34	0.85	0.55-1.32	0.89	0.59-1.34
HDM[b]	0.57	0.10-3.32	0.76	0.30-1.93	**0.47**	**0.23-0.96**	0.52	0.27-1.00
Mugwort	0.91	0.21-3.87	0.79	0.40-1.56	0.78	0.44-1.38	0.78	0.46-1.34
Mold[c]	-	-	-	-	-	-	-	-
Egg	1.05	0.47-2.33	0.88	0.43-1.80	1.74	0.69-4.39	1.03	0.59-1.81
Milk	1.21	0.65-2.24	1.17	0.67-2.01	1.63	0.69-3.87	1.24	0.78-1.97
Fish[d]	-	-	-	-	-	-	-	-
Wheat	0.68	0.24-1.90	1.14	0.59-2.20	0.65	0.24-1.71	0.87	0.48-1.60
Peanut	**1.88**	**1.03-3.44**	1.61	0.95-2.74	1.03	0.54-1.96	1.46	0.89-2.40
Soy	0.87	0.31-2.43	1.45	0.74-2.82	1.17	0.56-2.42	1.18	0.64-2.20

[a]Logistic regression model using generalized estimating equations (GEEs), adjusted for sex, heredity and preschool eczema. Effect modification was tested for sex, heredity, breastfeeding, parental smoking during infancy, mother's age, socio-economy and preschool eczema
Children without *FLG* mutation (n=1753) were the reference

[b]House dust mite (HDM)

[c]None of the children with *FLG* mutation displayed IgE sensitization to mold at 4 and 16 years

[d]None of the children with *FLG* mutation displayed IgE sensitization to fish at 8 and 16 years

Significant differences in bold

TABLE E4

Proportions of children with IgE sensitization to common airborne and food allergens among children with any sensitization at the respective follow-up and data on *FLG* mutation.

	4 years (n=380)			8 years (n=664)			16 years (n=745)		
	FLG mutation n=30	No *FLG* mutation n=350	P value	*FLG* mutation n=50	No *FLG* mutation n=614	P value	*FLG* mutation n=55	No *FLG* mutation n=690	P value
Monosensitization	36.7%	48.0%	.23	40.0%	38.9%	0.88	32.7%	33.3%	0.93
Oligosensitization	43.3%	30.3%	.14	22.0%	27.7%	0.39	30.9%	27.5%	0.59
Polysensitization	20.0%	21.7%	.83	38.0%	33.4%	0.51	36.4%	39.1%	0.69
Cat	16.7%	24.3%	.35	32.0%	36.0%	0.57	43.6%	39.4%	0.54
Dog	20.0%	18.9%	.88	44.0%	32.9%	0.11	40.0%	48.7%	0.21
Horse	20.0%	13.4%	.32	28.0%	18.7%	0.11	29.1%	22.3%	0.25
Timothy	26.7%	24.6%	.80	38.0%	42.5%	0.54	60.0%	58.8%	0.87
Birch	46.7%	37.4%	.32	44.0%	44.5%	0.95	50.9%	51.3%	0.96
HDM[a]	3.3%	7.7%	.38	10.0%	12.2%	0.64	18.2%	29.0%	0.09
Mugwort	6.7%	6.0%	.88	20.0%	21.7%	0.78	27.3%	28.4%	0.86
Mold	0.0%	4.9%	.22	10.0%	6.7%	0.37	0.0%	4.8%	0.10
Egg	23.3%	20.6%	.72	18.0%	18.2%	0.97	9.1%	5.2%	0.23
Milk	36.7%	34.9%	.84	32.0%	27.9%	0.53	10.9%	6.5%	0.22
Fish	2.9%	3.3%	.88	0.0%	1.0%	0.48	0.0%	0.6%	0.57
Wheat	13.3%	16.9%	.62	20.0%	17.3%	0.62	7.3%	11.0%	0.39
Peanut	**43.3%**	**22.3%**	**.010**	**36.0%**	**21.8%**	**0.022**	16.4%	17.1%	0.89
Soy	13.3%	12.9%	.94	20.0%	13.7%	0.22	10.9%	11.3%	0.93

[a]House dust mite (HDM)

Significant differences in bold (P<.05)

TABLE E5

Cluster analysis on IgE sensitization at 4, 8, and 16 years. Descriptive analysis in a 2-cluster model among children with any IgE sensitization at each respective age.

	Cluster 1	Cluster 2	All	P-value	Missing
2-cluster model at 4 years	n=506	n=89	n=595		
Preschool eczema	**47.0%**	**80.9%**	**52.1%**	**<.001**	**0**
FLG mutation	7.8%	8.3%	7.9%	.89	215
Male sex	**52.8%**	**68.5%**	**55.1%**	**.006**	**0**
Heredity[a]	49.4%	54.0%	50.1%	.43	6
Parental smoking[b]	**21.0%**	**32.2%**	**22.6%**	**.021**	**3**
Exclusive breastfeeding ≥4 months	78.0%	78.4%	78.0%	.93	3
Monosenstization	**58.9%**	**0.0%**	**50.1%**	**<.001**	**0**
Oligosensitization	**35.6%**	**0.0%**	**30.3%**	**<.001**	**0**
Polysensitization	**5.5%**	**100.0%**	**19.7%**	**<.001**	**0**
2-cluster model at 8 years	n=564	n=241	n=805		
Preschool eczema	**41.1%**	**66.0%**	**48.6%**	**<.001**	**0**
FLG mutation	6.7%	9.6%	7.5%	.20	141
Male sex	52.5%	57.3%	53.9%	.21	0
Heredity[a]	**48.0%**	**61.8%**	**52.2%**	**<.001**	**4**
Parental smoking[b]	20.8%	20.2%	20.6%	.84	5
Exclusive breastfeeding ≥4 months	78.6%	80.3%	79.2%	.59	4
Monosenstization	**55.1%**	**0.0%**	**38.6%**	**<.001**	**0**
Oligosensitization	**39.7%**	**0.0%**	**27.8%**	**<.001**	**0**
Polysensitization	**5.1%**	**100.0%**	**33.5%**	**<.001**	**0**
2-cluster model at 16 years	n=663	n=471	n=1134		
Preschool eczema	**38.8%**	**54.6%**	**44.2%**	**<.001**	**0**
FLG mutation	8.4%	6.1%	7.4%	.22	389
Male sex	53.2%	54.8%	53.9%	.61	0
Heredity[a]	**48.0%**	**58.2%**	**52.2%**	**.001**	**11**
Parental smoking[b]	21.1%	18.3%	19.9%	.20	6
Exclusive breastfeeding ≥4 months	79.2%	77.9%	78.7%	.17	9
Monosenstization	**61.4%**	**0.0%**	**35.9%**	**<.001**	**0**
Oligosensitization	**35.6%**	**14.0%**	**26.6%**	**<.001**	**0**
Polysensitization	**3.0%**	**86.0%**	**37.5%**	**<.001**	**0**

[a]Mother and/or father with doctor's diagnosis of asthma and/or hay fever and/or eczema (contact allergy among parents is excluded) at baseline
[b]Any of the parents smoked at least one cigarette per day at birth
Significant differences in bold