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Filaggrin loss-of function mutations are associated with food allergy in childhood and adolescence

Devasmitha Venkataraman, MD, MRCPCH, MSc Allergy[1,2], **Nelís Soto-Ramírez, MPH, MD**[3], **Ramesh J Kurukulaaratchy, DM, FRCP**[4,5], **John W Holloway, PhD**[6,7], **Wilfried Karmaus, MD, Dr. med, MPH**[3], **Susan L Ewart, PhD**[8], **S Hasan Arshad, FRCP, DM**[4,5,6], and **Mich Erlewyn-Lajeunesse, FRCPCH, DM**[5,6]

[1]University of Southampton, Faculty of Medicine, Southampton, UK

[2]South Tees Hospitals NHS Foundation Trust, Memphis Tennessee, USA

[3]Division of Epidemiology, Biostatistics, and Environmental Health, School of Public Health, University of Memphis, Memphis Tennessee, USA

[4]David Hide Asthma and Allergy Research Centre, Isle of Wight, UK

[5]University Hospitals Southampton NHS Foundation Trust, Southampton, UK

[6]Clinical and Experimental Sciences, University of Southampton, UK

[7]Human Development and Health, Faculty of Medicine, University of Southampton, UK

[8]College of Veterinary Medicine, Michigan State University, East Lansing, Michigan, USA

Abstract

Background—Filaggrin is an epidermal protein that has a role in skin barrier function. Filaggrin loss-of-function *FLG-LOF*) mutations are a significant risk factor for eczema and atopy, but their association with food allergy (FA) is less clear.

Objective—We explored the longitudinal relationship between three common *FLG*-LOF mutations and FA using the Isle of Wight birth cohort.

Correspondence and reprint requests to: Professor S. Hasan Arshad S.H.Arshad@soton.ac.uk, Tel: +44 (0)2380795756; Fax: +44 (0)2380878847.

Contributors

MEL generated the original hypothesis and all authors contributed to study design. SHA and RJK were responsible for all allergy phenotype data collection and SLE for genetic data collection. WK, SHA and JWH advised on analysis and interpretation of the genetic data. DV collated, analysed and interpreted the food allergy data and NSR analysed the path analysis. DV wrote the first draft of the manuscript, and all authors have seen and approved the final version of the report. MEL and SHA will serve as guarantors for its contents.

Conflict of interest statement

None of the authors have any conflicts of interests to declare.

Methods—FA diagnosis was based on recognised allergic reactions within 4 hours following exposure to known food allergens. Food allergen sensitization (FAS) was identified by skin prick test conducted between 1–18 years to a range of food allergens. Three filaggrin mutations were genotyped in 1150/1456 children (79%). The temporal relationships between FA, FAS and eczema in children with filaggrin mutations were explored using path analysis with total, direct and indirect effect models.

Results—There was a significant total effect of *FLG*-LOF mutations on the risk of FA in later childhood at ages 10 (OR: 31.46, 95%CI 2.86, >100) and 18 years (OR: 4.25, 95%CI 1.55, 11.61). Path analysis showed that there was no direct effect of *FLG*-LOF mutations on FA at any age, however an indirect effect was found on FA at all ages via eczema and FAS in the earlier years

Conclusion—*FLG*-LOF mutations are associated with FA in older children via eczema and FAS in their early childhood. Our results highlight a biologically plausible pathway, which suggests that skin barrier function is important in the development and persistence of FA.

Keywords

Food Allergy; Filaggrin; *FLG*-LOF; Food allergen sensitization; Path analysis; Prediction; Eczema

Introduction

The filaggrin *(FLG)* gene encodes a key epidermal protein (*fil*ament-*agg*regating prote*in*), which plays a crucial role in maintaining the integrity of the skin (1–3). Loss-of-function mutations within the filaggrin gene (*FLG*-LOF) lead to reduced protein expression resulting in epidermal barrier dysfunction, making the skin more permeable to environmental allergens and increasing trans-epidermal water loss (4–6). The role of *FLG*-LOF variants in eczema has re-kindled the interest in the role of skin barrier dysfunction in the development of allergies.

According to the hypothesis proposed by Lack et al. (7, 8), exposure to food allergens by an oral route leads to tolerance, whereas cutaneous exposure leads to allergy. Animal studies have shown that allergen sensitization can occur via the cutaneous route via antigen presenting cells in the epidermis (9, 10). This occurs especially in the *FLG*-deficient state, and sensitization may be an important precursor to food and respiratory allergies. *FLG*-LOF mutations have been identified as a risk factor for allergic sensitization, atopic eczema and allergic rhinitis and asthma (only in the context of eczema), but their impact on food allergy has not yet been widely explored (11–15). To date, only one study has shown a significant association between *FLG*-LOF mutations and food allergy, which was limited to peanut allergy(16). Further studies are needed to corroborate the strength and consistency of the association and investigate this relationship with other types of food allergies.

We have previously investigated the time order sequence between *FLG*-LOF mutation, eczema and allergic sensitization (17), using the Isle of Wight (IOW) cohort, which showed that a combination of *FLG*-LOF and allergic sensitization in early life increases the risk of eczema in subsequent years (17). However, there have been no longitudinal studies

exploring the interplay and time order relationships between *FLG*-LOF mutations, food allergy (FA), food allergen sensitization (FAS), and eczema. This information is vital in understanding the development of food allergies and to guide the evolution of strategies to restore skin barrier and prevent the development of sensitization and food allergies.

Methods

The Isle of Wight (IOW) birth cohort is an un-selected, whole population birth cohort established in 1989, and has been followed up prospectively for 18 years, with the aim of studying the natural history of allergic diseases and the influence of genetic and environmental factors on the development and progression of allergies (17–20). The study was approved by the local research ethics committee (06/Q1701/34). All children (n=1536) consecutively born on the Isle of Wight, UK between 1 January 1989 and 28 February 1990 were enrolled in the study and 1456 were available for further follow-up. The children were assessed at 1 year (n=1374, 94.4%), 2 (n=1231, 84.5%), 4 (n=1218, 83.7%), 10 (n=1373, 94.3%), and 18 years (n=1313, 90.2%).

Diagnostic criteria for food allergy

Definition of FA—We applied an *a priori* definition of food allergy based on the following criteria (Study Criteria):

1. A reaction to a recognised food allergen as defined by the European Union(21) and the Committee on toxicity of chemicals in foods, consumer products and the environment(22) (e.g. Cow's Milk, Hen's Egg, wheat, Soya, Peanuts, Other nuts, Fish, and Shell Fish).
2. The report of recognised allergic symptoms(23) such as :
    a. localised symptoms: itching, sting/ burning of the lips/ mouth or throat, urticaria/ hives, angioedema
    b. abdominal: nausea, vomiting, crampy/ colicky abdominal pain, diarrhoea
    c. respiratory: wheeze, stridor, watery rhinitis, redness of eyes/ nose
    d. skin: urticaria, itching, flushed skin, worsening eczema
    e. systemic reaction: anaphylaxis
3. Temporal relationship: symptoms developing within 4 hours of food ingestion.

If criteria 1, 2, and 3 were fulfilled, children were designated as having a FA. Children with FA were further stratified based on the SPT results to the defined food allergen into subgroups: (FA+SPT Positive/ FA +SPT Negative, and FA + SPT Not available).

Eczema

Eczema was diagnosed based on the Hanifin and Rajka (24) criteria: itchy dermatitis lasting more than 6 weeks with characteristic morphology and distribution.

Food allergen sensitization (FAS

The skin prick test (SPT) was performed using standardised method and extracts (Alk-Abello, Horsholm, Denmark), towards a panel of common aero-allergens and food allergens. Food allergens included cows' milk, hen's egg, wheat, soya, cod and peanut. Food allergen sensitization (FAS) was defined as a positive reaction to one or more food allergens with a mean wheal diameter being ≥ 3mm greater than the negative control at 15 minutes. SPT was performed at 1 and 2 years in symptomatic children only and at 4, 10, and 18 years in all consenting participants.

Filaggrin genotyping

The *FLG* gene status was determined following extraction of DNA from the peripheral blood or saliva samples. Five polymorphisms (R501X, 2282del4, S3247X, 3702delG, and R2447X) leading to loss-of-function (LOF), prevalent in European populations, were genotyped as previously described (17). Children were classified as having *FLG*-LOF defect if they carry the minor allele for at least one of the 3 following *FLG* null variants: R501X, 2282del4, or S3247X.

Statistical analysis

SPSS (Version 19, IBM, USA) was used to prepare frequency tables and assess the prevalence of FA and FAS at each time point (1, 2, 4, 10 and 18 years). Significance of changes in FA prevalence rates over time (1, 4, 10 and 18 years) was tested using McNemar's test for paired data and χ^2/Fishers exact test for independent data. Path analysis (25) was used to explore the pathways leading to the development of FA in filaggrin-deficient individuals. We assessed the structure among multiple variables including allergic phenotypes such as eczema and FAS and decomposed the effects into total, direct, indirect effects (Mplus version 6) (26). In addition, we conducted separate pathways for FA and FAS. The detection of direct effects indicates the impact of a risk factor on an outcome that is not mediated by other variables. In contrast, indirect associations depicted the effect of a risk factor (X) on an outcome variable (Z) via an intervening variable (Y) such that X → Y → Z. The total effect of a risk factor is the combination of direct and indirect statistical relationships.

Results

The study population of the IOW birth cohort was dynamic, as many children participated at varying stages of the study, and not all children were seen at each time point (1, 2, 4, 10 and 18 years). Appendix 1(online repository) graphically depicts the availability of information regarding FA, FAS, and eczema at various stages of the study from 1 to 18 years. Since the 1 year and 2 year follow-up data on eczema and food allergy were collected in a relatively small time window, we have combined them for analytic purposes (Eczema 1 &2 years, FA 1&2 years).

Filaggrin Gene Analysis

The *FLG* genotype was determined in 1150 children (79%) of the cohort at 18 years. There were no significant differences between the characteristics (sex, eczema status and FA

status) of the whole population and the genotyped population (Appendix 2, online repository). The overall *FLG*-LOF mutation frequency was 10.3% as reported previously (17).

Food allergy and *FLG-LOF* mutations

We used logistic regression analysis in the initial assessment of relationship between *FLG*-LOF mutations and FA at 5 time points. We found a significant association between *FLG*-LOF mutations and FA in the whole population IOW cohort at 10 years (OR: 2.9, 95%CI (1.2, 7.0), Fishers Exact p= 0.022) and 18 years (OR: 2.5, 95%CI (1.2, 5.3) FE p=0.032) Figure 1) but not at 1, 2 and 4 years.

Table 1 provides information on the prevalence of FA, FAS and eczema between 1–18 years. The longitudinal trend in prevalence of FA in the IOW cohort show relatively constant prevalence rates in early childhood, (5.3%, 4.4% and 5% at 1, 2, and 4 years, respectively), with a significant decline at 10 years (2.3%, p<0.001), followed by a significant rise at 18 years (4.1%, p=0.02) (Table 1). The association between *FLG*-LOF mutations and FA corresponds to these points of significant change in FA prevalence. No significant associations were seen in the earlier years. A significant increase in FAS was also seen at 18 years.

Path analysis

We used path analysis (total, direct and indirect effects) to explore if the association between *FLG*-LOF mutations and FA was a direct effect, or it was an indirect effect secondary to the occurrence of eczema, or FAS. The relationship between *FLG*-LOF and FAS was not explored at 1 &2 years of age as only symptomatic children underwent skin prick testing and FAS data was not available in the whole cohort.

FLG-LOF, eczema, FAS, and FA pathways

We found a significant total effect of *FLG*-LOF on FA at ages 10 years (OR: 31.46, 95% CI (2.86, >100) p = 0.005, and 18 years (OR: 4.25, 95% CI (1.55, 11.61), p = 0.005); after adjusting for gender (Figure 2). This significant association was not seen in the earlier years. We also found significant associations between *FLG*-LOF and FAS at 4 years (OR 4.23, 95% CI (1.3, 13.74) p=0.01); 10 years (OR 7.24, 95% CI (1.83, 28.7) p=0.005) and 18 years (OR 2.75, 95% CI (1.17, 6.45), p=0.01).

Eczema at 1&2 years of age was associated with a total effect on FA at age 4 (OR 6.04, 95% CI (1.25, 29.9), p<0.0001) and FAS at ages 4 (OR 20.1, 95% CI (4.40, 54.50), p<0.0001) and 18 years (OR 18.2, 95% CI (5.47, 65.3), p<0.0001). In addition, eczema at age 4 was linked to FAS at ages 10 (OR 6.9, 95% CI (3.65, 13.04), p<0.0001) and 18 years (OR 3.82, 95% CI (2.44, 5.99), p<0.0001).

No direct effect of *FLG*-LOF on FA or FAS was found across all ages (Figure 3). A direct association was found between *FLG-LOF* with eczema at 1&2 years of age (OR 1.79, 95% CI (1.11, 2.88); p = 0.01) and 4 years (OR 1.78, 95% CI (0.98, 3.21); p= 0.05). Eczema at 1–2 was directly associated with food allergy at 1&2 years (OR 5.46, 95% CI (2.95, 10.08);

p<0.0001) and 4 year (OR 2.36, 95% CI (1.24, 4.48); p=0.01) and FAS at age 4 years (OR: 2.88, 95% CI (1.25, 6.62); p=0.01) in *FLG-LOF* variant individuals. Eczema at 4 years was directly associated with FAS at 10 years (OR 4.40, 95%CI (1.95, 9.92); p<0.0001,) and 18 years (OR: 2.01, 95% CI 1.09, 3.68) p=0.01) in *FLG-LOF* variant individuals. Also, we found a direct effect of FAS at age 4 years on FA at age 10 years (OR 7.85, 95% CI (2.02, 30.52); p = 0.003). FAS at age 10 years was associated with FA at age 18 years (OR 4.88. 95% CI (1.68, 14.10); p = 0.001).

Early eczema in individuals with a *FLG*-LOF variant had an indirect effect on FAS and FA (Table 2). There was an indirect effect of *FLG*-LOF mutations through eczema at 1–2 years of age on FA at 1&2 years (OR 2.81, 95% CI (1.15, 6.86); p=0.02), FA at 4 years (OR 15.48, 95% CI (1.32, >100); p=0.02) and on FAS at 4 years (OR 2.18, 95% CI (0.99, 4.76); p=0.05). Further, *FLG*-LOF variants had an indirect effect on FA at age 10 (OR: 10.0; p = 0.03, CI 1.14, 87.0) through the occurrence of eczema at 1–2 years of age and FA at age 4 years. *FLG*-LOF mutation had an indirect effect via eczema at 4 years on FAS at 10 years (OR 4.49, 95% CI (1.44, 13.99); p=0.01) and 18 years (OR 2.38, 95%CI (1.19, 4.74); p=0.01), and FA at 18 years (OR: 21.93; 95% CI (1.50, >100); p = 0.02).

Discussion

This is the first study to explore the relationship between *FLG*-LOF mutations and the longitudinal trends in FA. Our study showed that *FLG-LOF* mutations were associated with FA and FAS at 10 and 18 years (total effects). Further exploration via path analysis suggested an association between FLG-LOF and eczema in younger children and the progression to FAS and FA in older children.

Filaggrin and food allergy

This is the first study to associate *FLG*-LOF with all causes of FA rather than with a specific food allergen. Brown SJ *et al* (16) described an association of *FLG-LOF* and peanut allergy in three different populations. Their study supports a relationship of FLG-LOF with peanut FA, but does not consider other types of FA. Our study demonstrated a significant association (total effect via path analysis) between FA and FLG-LOF mutations in older children and young adults (i.e., 10 and 18 years), but not during the earlier years. FA in early childhood are often due to egg and milk allergy, which tends to improve, while in older children and young adults, peanut and sea food allergies become more prevalent and tend to persist (Appendix 3, online repository). Our findings therefore further confirm the association of *FLG*-LOF with more persistent forms of food allergy

FLG-LOF Pathway analysis

The two most common pathways detected in this study are the following: (1) *FLG-LOF* mutations→Eczema→Food sensitization; (2) *FLG-LOF* mutations→Eczema→Food allergy. We found that FAS has a direct relationship with food allergy and the complex interplay between eczema and FAS in the pathway increases the odds of food allergies significantly in later life in filaggrin deficient individuals (*FLG*-LOF mutations→Eczema→Food allergen sensitization→Food allergy). These are biologically plausible pathways for a relationship

between *FLG-LOF* and FA and are in keeping with current hypotheses on causality (7). This suggests that two different mechanistic pathways may be sequentially involved in the pathogenesis of FA: a barrier dysfunction caused by *FLG*-LOF and a barrier defect with associated inflammation caused by eczema leading to subsequent sensitization and immune response, all increasing the odds of developing FA at different time points (Fig 3, 4, and 5).

These results suggest a role for FLG-LOF mutations in the development of FA. The barrier defect associated with *FLG* deficient individuals makes them susceptible to development of cutaneous sensitization via antigen presenting cells and systemic atopic response (27–29). Peanut sensitization may occur via the topical application of peanut oil to the skin (8), and cutaneous exposure via presence of peanut allergen in the environment (30), and this process may be enhanced by a deficient barrier caused by *FLG-LOF* mutations or eczema (8). Animal studies also suggest that allergic sensitization can occur in the absence of cutaneous inflammation / eczema via the filaggrin deficient skin (10, 31).

Marenholz et al (11) reported that associations between *FLG-LOF* mutations and allergic sensitization/asthma were significant only in the presence of eczema. This supports the notion that presence of both inflammation and a defective barrier in eczema lead to the transcutaneous exposure to allergens and their subsequent sensitization and development of allergic disease. Further work by Marenholz et al (11) showed that in the presence of eczema and food sensitization, *FLG-LOF* mutation strongly predicted the development of childhood asthma, suggesting synergistic interaction between *FLG-LOF* variants and food sensitization, leading to the transition of allergic phenotype from eczema to asthma. These studies suggest that eczema plays an early and significant role in the progression of allergic phenotypes involved in the allergic march.

Ziyab et al (17), undertook a temporal sequence analysis to ascertain the time order sequence between eczema and allergic sensitization (both food and aero-allergens) with respect to *FLG*-LOF mutations in the IOW cohort. They found that *FLG*-LOF mutations and eczema increased the risk of subsequent allergic sensitization only in the first 10 years of life. Our study has looked into the complex interactions specifically looking at FAS and FA, and the results show that the risk of food sensitization is increased beyond 10 years via eczema in *FLG*-LOF mutations. *FLG*-LOF mutations has an indirect effect on FAS and FA in later childhood via the occurrence of eczema in earlier years, and this needs to be further explored by other longitudinal studies.

Flohr et al examined the relationship between *FLG*-LOF, atopic dermatitis, trans-epidermal water loss and food allergic sensitization in infancy (32). Their study found that children with eczema are more likely to be sensitized to food allergens independently of *FLG-LOF* status, severity of eczema and trans-epidermal water loss. They did not show a relationship between *FLG-LOF* and food sensitization, but instead severity of eczema was associated with FAS. Our study also showed a strong association between eczema (1&2 years, 4 years) and FAS at 4, 10 and 18 years, and lack of relationship between *FLG*-LOF and FA in the early years. The association between *FLG*-LOF and FA only becomes apparent in later childhood and adolescence. The effect of *FLG*-LOF may be important in the maintenance of food allergy but may play less of a role in FA or FAS events in infancy, where atopic

dermatitis may adversely affect the skin barrier and contribute to allergic sensitisation and later development of FA in *FLG* deficient children

In summary, *FLG*-LOF mutations have an indirect effect on FA via eczema and FAS in the pathway. Our findings have discerned a relationship between *FLG*-LOF, eczema, FAS and FA. They provide further insight into the role of the skin barrier in the pathogenesis of FA.

Strengths and limitations

All consecutive children born in 1989 were enrolled into this birth cohort and there was no selection bias at recruitment. Data were gathered prospectively and the overall follow-up participation rates were high throughout the study period (84–94.3%), which ruled out a major bias due to loss-to-follow-up. The prevalence of eczema and allergic sensitization did not differ between the genotyped and full cohort data, supporting generalizability of the study findings. A unique aspect of our study is the repeated longitudinal assessment of individuals throughout childhood, where each child acts as their own control.

The Isle of Wight birth cohort is dynamic; some children did not participate at one time point, but re-joined at another. For this reason comparisons were made between two time points, where FA information on an individual was available at both time points (1–2, 2–4, 4–10, 10–18 years). We further explored this in a stable cohort where FA data was available at all five time points (stable cohort, Appendix 4, online repository) and our findings were similar.

In our study, strict symptom-based criteria were used to diagnose FA, as is common in a clinical setting. This method of diagnosis of FA is superior to FA surveys used in other population studies, but certainly not as accurate as studies using the ‘gold standard’ of double blind food challenges.

The measurement of SPT was performed at key stages, allowing FAS to be treated as a time-dependant covariate. The proportion of children that underwent SPT were 67.4%, 71.1% and 58.5% at 4, 10 and 18 years, respectively, which were much lower than the proportion of children that were followed-up at the same time periods. This reduced the availability of food sensitization data. We also only had information on SPT’s in symptomatic children at age 1 and 2, which limited the assessment of the relationship between allergic sensitization and food allergy in the early stages of childhood.

Conclusions

Our study is the first to investigate the relationship between *FLG*-LOF mutations, food allergen sensitisation, and food allergy longitudinally over 18 years (five exams), with each child acting as their own control in this process. We found statistically significant total effects of *FLG-LOF* mutations on FA at ages 10 and 18 years but not in early childhood, suggesting that *FLG-LOF* mutations may be associated with more persistent forms of FA in older children and young adults. We further explored this association via path-analysis by investigating effects of eczema and FAS in this pathway. *FLG*-LOF mutations had an indirect effect on FA in childhood by the occurrence of eczema and FAS in earlier years.

Impaired skin barrier function as a result of both eczema and FLG-LOF seems to be a crucial common factor in the pathogenesis of food allergy and improvement in the barrier function of skin in early childhood may influence the further development of allergy. This study has helped improve the understanding of pathways leading to FA which will help to develop targeted preventive and disease modifying strategies.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations used

CI	Confidence Interval
FA	Food Allergy
FAS	Food Allergen Sensitization
FLG	*Filaggrin*
***FLG*-LOF**	Filaggrin Loss-of-function
IOW	Isle of Wight
SPSS	Statistical product and service solutions
SPT	Skin Prick Test

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Clinical Implications/ Key messages

Our study demonstrates the complex interactions between eczema, food sensitization and food allergy over time and the direct and indirect pathways predisposing to food allergy in individuals with filaggrin gene (*FLG*) variants. The associations (total effect) between *FLG*-loss of function (*FLG*-LOF) mutations and food allergy are stronger at 10 and 18 years than at earlier ages, suggesting that *FLG*-LOF mutations may be associated with more persistent forms of childhood food allergy. Pathway analysis showed significant and consistent relationship between filaggrin-loss of function, eczema and allergic sensitization to food in early years and food allergy in later childhood. This pathway provides a biologically plausible mechanism for the role of *FLG* in FA.

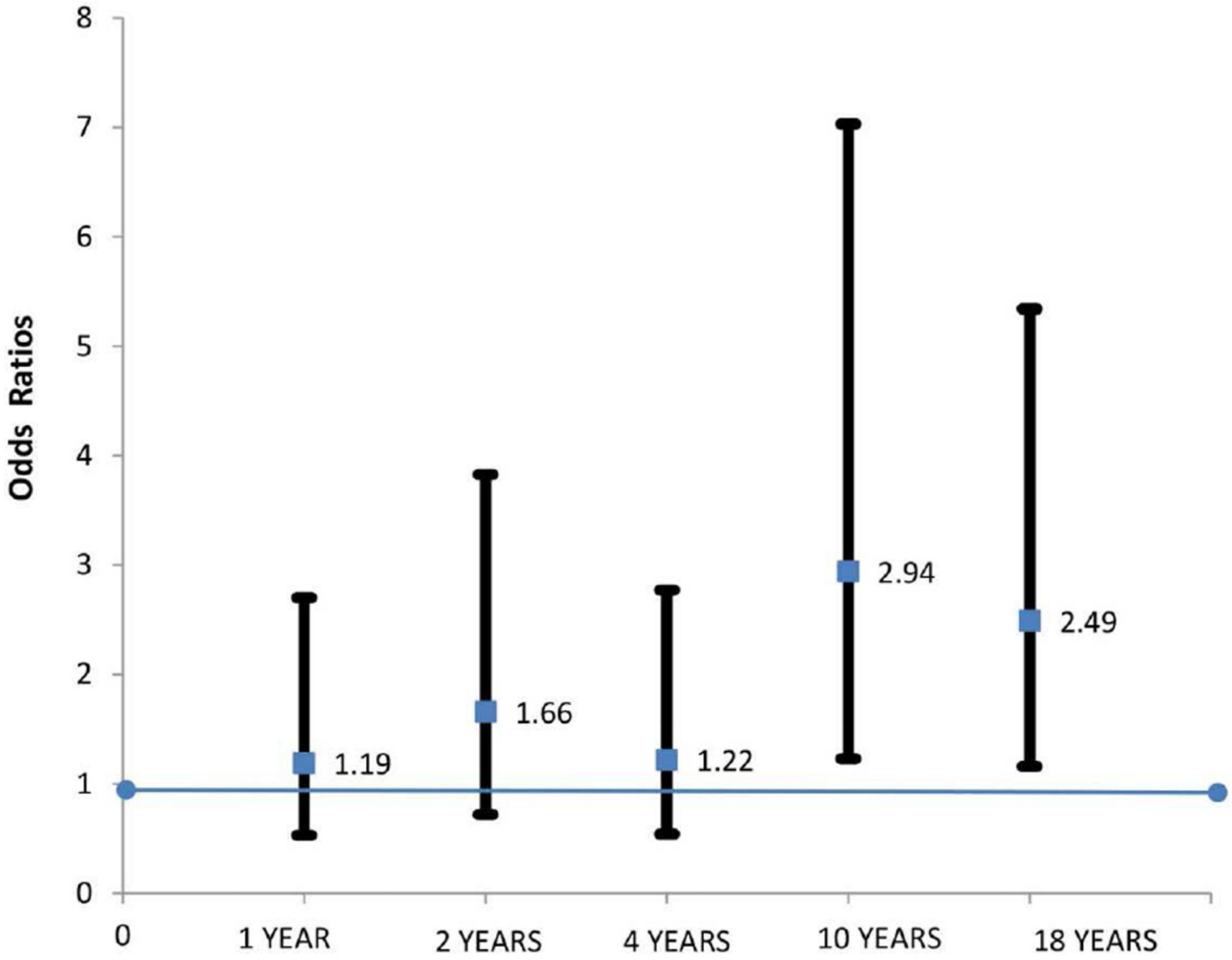

Figure 1.

Odds of developing food allergy in children with FLG-LOF mutation at 1, 2, 4, 10 and 18 years of age. A significant association seen between FLG-LOF mutation and FA at ages 10 and 18 years (logistic regression)

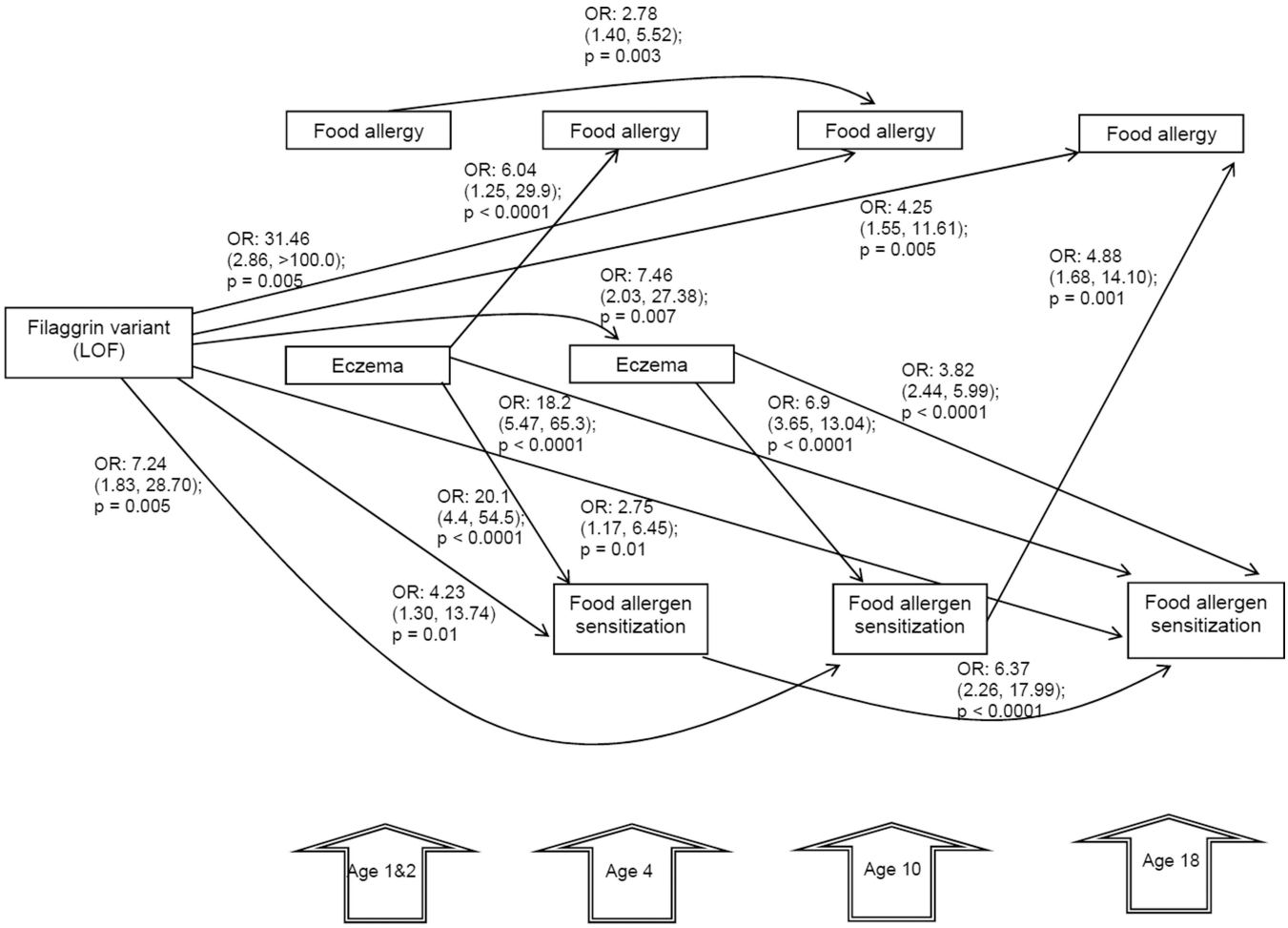

Figure 2.

Analytical path model exploring the total effects of *FLG*-LOF, eczema at ages 1&2 and 4 years on food allergy (FA) and food allergy sensitization (FAS) at 4, 10, and 18 years of age. The path coefficient (total effects) represents the Odds Ratios. Goodness of fit adjusted for degrees of freedom: 0.99; comparative fit index: 0.99; root mean square error of approximation: ≤ 0.06; deviation between covariance structure and the empirical covariance: Chi-squared/degree of freedom ≤2; $p<0.05$. Only significant direct paths are shown. The relationship between *FLG*-LOF and FAS was not explored at 1 &2 years of age as only symptomatic children underwent skin prick testing and FAS data not available in whole cohort.

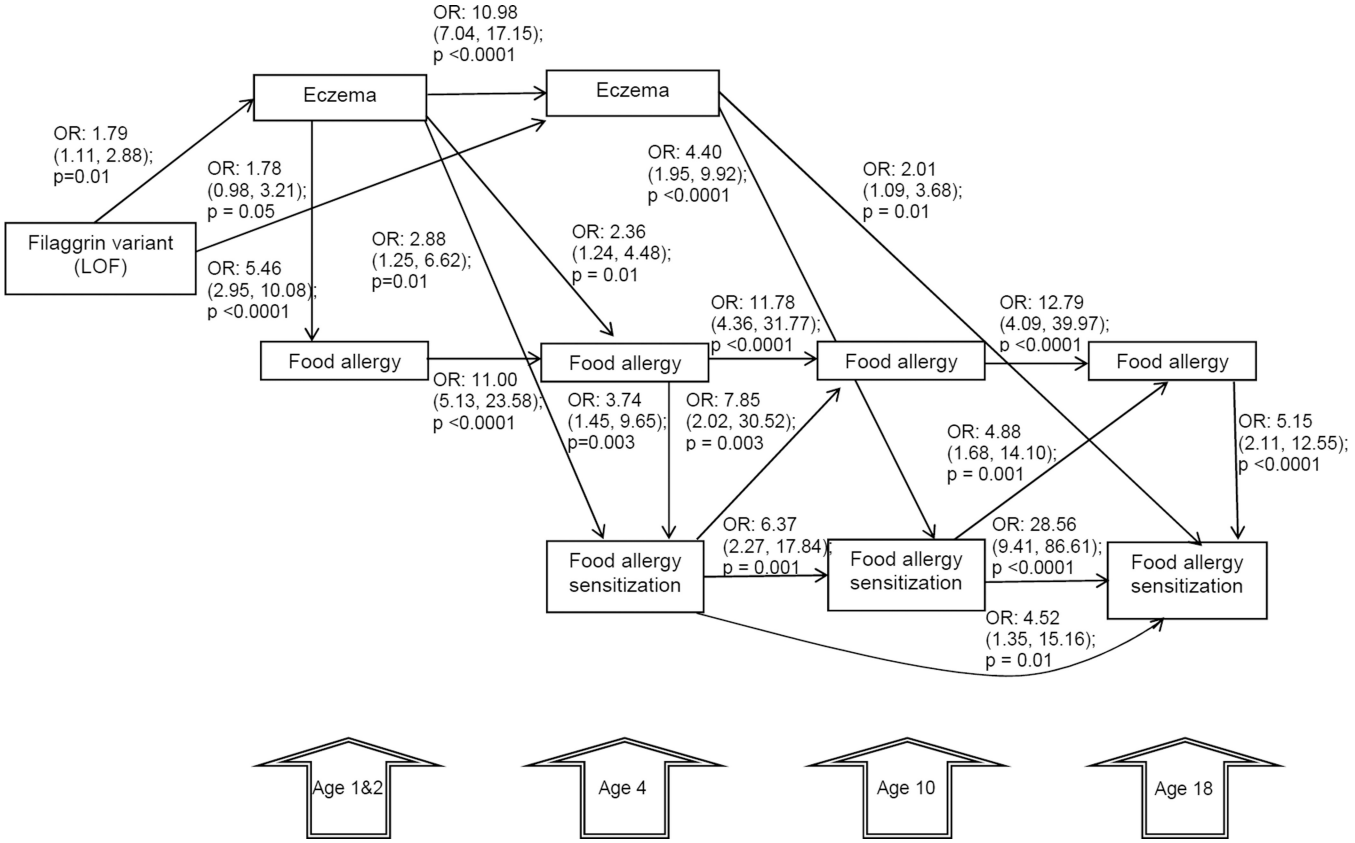

Figure 3.

Analytical path model exploring the direct effects of *FLG*-LOF, eczema at ages 1& 2 and 4 years on food allergy (FA) and food allergy sensitization (FAS) at 4, 10, and 18 years of age. The path coefficient (direct effects) represents the Odds Ratios. Goodness of fit adjusted for degrees of freedom: 0.99; comparative fit index: 0.99; root mean square error of approximation: ≤ 0.06; deviation between covariance structure and the empirical covariance: Chi-squared/degree of freedom ≤2; $p<0.05$. Only significant direct paths are shown. The relationship between *FLG*-LOF and FAS was not explored at 1 &2 years of age as only symptomatic children underwent skin prick testing and FAS data not available in whole cohort.

Table 1

Prevalence of Food Allergy, Food allergen sensitisation and Eczema in the study population over 18 years

Age	FA based on Study Criteria %, (95% CI)	Prevalence of Food Allergen Sensitisation (based on SPT) %, (95% CI)	Prevalence of Eczema %, (95% CI)
1 year	5.3 (4.2–6.7)%	**	11.7 (9.9–13.5)%
2 years	4.4 (3.4–5.7)%	**	19.0(16.7–21.2)%
4 years	5.0 (3.9–6.4)%	3.2 (2.1–4.3)%	12.1(10.3–13.9)%
10 years	2.3 (1.7–3.3)%* (p<0.001, Significant drop in prevalence)	4.5 (3–5.4)%	13.7(11.9–15.5)%
18 years	4.1(3.2–5.4)%* (p=0.024, Significant increase in prevalence)	21.4(18.6–24.2)% * (p<0.001, Significant increase in sensitization)	12.1(10.3–13.9)%

** SPT at 1 and 2 years limited to symptomatic children

Table 2

Significant results from indirect pathway analysis

FLG status	1 & 2 years	4 years	10 years	18 years	OR[a] (95% CI)	P value
FLG LOF →	Eczema → **Food allergy**[b]				2.81 (1.15, 6.86)	0.02
FLG LOF →	Eczema →	**Food allergy**[b]			15.48 (1.32, >100)	0.02
FLG LOF →	Eczema →	**Food sensitization**[b]			2.18 (0.99, 4.76)	0.05
FLG LOF →	Eczema →	Food allergy →	**Food allergy**[b]		10.0 (1.14, 87.0)	0.03
FLG LOF →	→	Eczema →	**Food sensitization**[b]		4.49 (1.44, 13.99)	0.01
FLG LOF →	→	Eczema →	→	**Food sensitization**[b]	2.38 (1.19, 4.74)	0.01
FLG LOF →	→	Eczema →	Food sensitization →	**Food allergy**[b]	21.9 (1.50, >100.0)	0.02

Only significant indirect paths are shown. Other models of the relationship between FLG-LOF mutations, eczema, food sensitization and food allergy were tested but did not have significant outcome.

[a] OR represents the indirect association of FLG LOF on food allergy and food sensitization at different ages.

[b] The outcomes of the indirect paths are in bold.