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

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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 3 common *FLG*-LOF mutations and FA using the Isle of Wight birth cohort.

Methods: FA diagnosis was based on recognized allergic reactions within 4 hours after exposure to known food allergens. Food allergen sensitization (FAS) was identified by using skin prick tests conducted between 1 and 18 years of age to a range of food allergens. Three *FLG* mutations were genotyped in 1150 (79%) of 1456 children. The temporal relationships between FA, FAS, and eczema in children with *FLG* mutations were explored by 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 the ages of 10 (odds ratio, 31.46; 95% CI, 2.86 to >100) and 18 (odds ratio, 4.25; 95% CI, 1.55-11.61) years. 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 through eczema and FAS in the earlier years.

Conclusion: *FLG*-LOF mutations are associated with FA in older children through eczema and FAS during early childhood. Our results highlight a biologically plausible pathway, which suggests that skin barrier function is important in the development and persistence of FA. (J Allergy Clin Immunol 2014;134:876-82.)

Key words: *Food allergy, filaggrin,* FLG-*LOF, food allergen sensitization, path analysis, prediction, eczema*

The filaggrin gene *(FLG)* encodes a key epidermal protein (filament-aggregating protein) that plays a crucial role in maintaining skin integrity.[1-3] *FLG* loss-of-function (*FLG*-LOF) mutations lead to reduced protein expression, resulting in epidermal barrier dysfunction and making the skin more permeable to environmental allergens and increasing transepidermal water loss.[4-6] The role of *FLG*-LOF variants in patients with eczema has rekindled interest in the role of skin barrier dysfunction in the development of allergies.

According to a hypothesis proposed by Lack et al,[7,8] exposure to food allergens through the oral route leads to tolerance, whereas cutaneous exposure leads to allergy. Animal studies have shown that allergen sensitization can occur through the cutaneous route as a result of antigen-presenting cells in the epidermis.[9,10] This occurs especially in the *FLG*-deficient state, and sensitization might 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 effect on food allergy (FA) has not yet been widely explored.[11-15] To date, only 1 study has shown a significant association between *FLG*-LOF mutations and FA, 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 mutation 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, FA, food allergen sensitization (FAS), and eczema. This information is vital in understanding the development of food allergies and in guiding the evolution of strategies to restore the skin barrier and prevent the development of sensitization and food allergies.

METHODS

The IOW birth cohort is an unselected, whole population birth cohort established in 1989 that has 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

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Abbreviations used
FA: Food allergy
FAS: Food allergen sensitization
FLG: Filaggrin
FLG-LOF: Filaggrin loss-of-function
IOW: Isle of Wight
OR: Odds ratio
SPT: Skin prick test

born on the IOW, United Kingdom, between January 1, 1989, and February 28, 1990, were enrolled in the study, and 1456 were available for further follow-up. The children were assessed at 1 (n = 1374 [94.4%]), 2 (n = 1231 [84.5%]), 4 (n = 1218 [83.7%]), 10 (n = 1373 [94.3%]), and 18 (n = 1313 [90.2%]) years of age.

Diagnostic criteria for FA

We applied an *a priori* definition of FA based on the following criteria (study criteria):

1. a reaction to a recognized food allergen, as defined by the European Union[21] and the Committee on Toxicity of Chemicals in Foods, Consumer Products and the Environment,[22] such as allergy to cow's milk, hen's egg, wheat, soya, peanuts, other nuts, fish, and shellfish;
2. a report of recognized allergic symptoms,[23] such as:
   A. localized symptoms: itching, sting/burning of the lips/mouth or throat, urticaria/hives, and angioedema;
   B. abdominal symptoms: nausea, vomiting, crampy/colicky abdominal pain, and diarrhea;
   C. respiratory symptoms: wheeze, stridor, watery rhinitis, and redness of eyes/nose;
   D. skin symptoms: urticaria, itching, flushed skin, and worsening eczema; and
   E. systemic reactions: anaphylaxis; and
3. temporal relationship: symptoms developing within 4 hours of food ingestion.

If criteria 1, 2, and 3 were fulfilled, children were designated as having FA. Children with FA were further stratified based on skin prick test (SPT) responses to the defined food allergen into subgroups: FA + positive SPT response/FA + negative SPT response and FA + unavailable SPT response.

Eczema

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

FAS

SPTs were performed with standardized methods and extracts (ALK-Abelló, Hørsholm, Denmark) to a panel of common aeroallergens and food allergens. Food allergens included cows' milk, hen's egg, wheat, soya, cod, and peanut. FAS was defined as a positive reaction to 1 or more food allergens with a mean wheal diameter of 3 mm or larger than that elicited by the negative control at 15 minutes. SPTs were performed at 1 and 2 years of age in symptomatic children only and at 4, 10, and 18 years of age in all consenting participants.

FLG genotyping

The *FLG* gene status was determined after extraction of DNA from peripheral blood or saliva samples. Five polymorphisms (R501X, 2282del4, S3247X, 3702delG, and R2447X) leading to loss of function (LOF) that are prevalent in European populations were genotyped, as previously described.[17] Children were classified as having an *FLG*-LOF defect if they carried the minor allele for at least 1 of the 3 following *FLG* null variants: R501X, 2282del4, or S3247X.

Statistical analysis

SPSS software (version 19; IBM, Armonk, NY) 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). The significance of changes in FA prevalence rates over time (1, 2, 4, 10, and 18 years) was tested by using the McNemar test for paired data and the χ^2/Fisher exact test for independent data. Path analysis[25] was used to explore the pathways leading to the development of FA in filaggrin-deficient subjects. We assessed the structure among multiple variables, including allergic phenotypes, such as eczema and FAS, and decomposed the effects into total, direct, and indirect effects (Mplus version 6).[26] In addition, we conducted separate pathways for FA and FAS. The detection of direct effects indicates the effect 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)* through an intervening variable *(Y)* as follows: 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 because 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 of age). Fig E1 in this article's Online Repository at www.jacionline.org depicts the availability of information regarding FA, FAS, and eczema at various stages of the study from 1 to 18 years. Because the 1- and 2-year follow-up data on eczema and FA were collected in a relatively small time window, we have combined them for analytic purposes (eczema, 1 and 2 years; FA, 1 and 2 years).

FLG gene analysis

The *FLG* genotype was determined in 1150 (79%) children of the cohort at 18 years of age. There were no significant differences between the characteristics (sex, eczema status, and FA status) of the whole population and the genotyped population (see Table E1 in this article's Online Repository at www.jacionline.org). The overall *FLG*-LOF mutation frequency was 10.3%, as reported previously.[17]

FA 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 (odds ratio [OR], 2.9; 95% CI, 1.2-7.0; $P = .022$, Fisher exact test) and 18 years (OR, 2.5; 95% CI, 1.2-5.3; $P = .032$, Fisher exact test; Fig 1) of age but not at 1, 2, and 4 years of age.

Table I provides information on the prevalence of FA, FAS, and eczema between 1 and 18 years of age. The longitudinal trend in prevalence of FA in the IOW cohort shows relatively constant prevalence rates in early childhood (5.3%, 4.4%, and 5% at 1, 2, and 4 years of age, respectively), with a significant decrease at 10 years (2.3%, $P < .001$) and a significant increase at 18 years (4.1%, $P = .02$) of age (Table I). The association between *FLG*-LOF mutations and FA corresponds to these points of

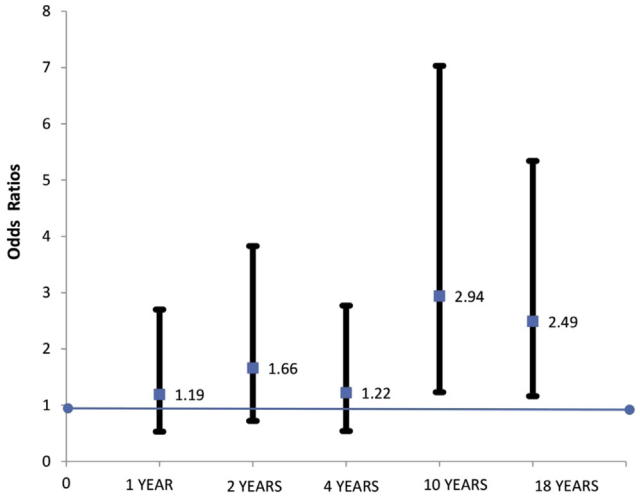

FIG 1. Odds of having FA in children with *FLG*-LOF mutations at 1, 2, 4, 10, and 18 years of age. A significant association was seen between *FLG*-LOF mutation and FA at ages 10 and 18 years (logistic regression).

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 of age.

Path analysis

We used path analysis (total, direct, and indirect effects) to explore whether the association between *FLG*-LOF mutations and FA was a direct effect or whether 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 and 2 years of age because only symptomatic children underwent SPTs and FAS data were not available in the whole cohort.

FLG-LOF, eczema, FAS, and FA pathways

Total effects model. We found a significant total effect of *FLG*-LOF mutation on FA at the ages of 10 (OR, 31.46; 95% CI, 2.86 to >100; $P = .005$) and 18 (OR, 4.25; 95% CI, 1.55-11.61; $P = .005$) years (Fig 2). This significant association was not seen in the earlier years. We also found significant associations between *FLG*-LOF and FAS at 4 (OR, 4.23; 95% CI, 1.3-13.74; $P = .01$), 10 (OR, 7.24; 95% CI, 1.83-28.7; $P = .005$), and 18 (OR, 2.75; 95% CI, 1.17-6.45; $P = .01$) years of age.

Eczema at 1 and 2 years of age was associated with a total effect on FA at age 4 years (OR, 6.04; 95% CI, 1.25-29.9; $P < .0001$) and FAS at ages 4 years (OR, 20.1; 95% CI, 4.40-54.50; $P < .0001$) and 18 years (OR, 18.2; 95% CI, 5.47-65.3; $P < .0001$). In addition, eczema at age 4 years was linked to FAS at ages 10 years (OR, 6.9; 95% CI, 3.65-13.04; $P < .0001$) and 18 years (OR, 3.82; 95% CI, 2.44-5.99; $P < .0001$).

Direct effect. No direct effect of *FLG*-LOF mutation on FA or FAS was found across all ages (Fig 3). A direct association was found between *FLG*-LOF mutation and eczema at 1 and 2 years of age (OR, 1.79; 95% CI, 1.11-2.88; $P = .01$) and 4 years of age (OR, 1.78; 95% CI, 0.98-3.21; $P = .05$). Eczema at 1 to 2 years of age was directly associated with FA at the ages of 1 and 2 years (OR, 5.46; 95% CI, 2.95-10.08; $P < .0001$) and 4 years (OR, 2.36; 95% CI, 1.24-4.48; $P = .01$) and FAS at age 4 years (OR, 2.88; 95% CI, 1.25-6.62; $P = .01$) in *FLG*-LOF variant subjects. Eczema at age 4 years was directly associated with FAS at age 10 years (OR, 4.40; 95% CI, 1.95-9.92; $P < .0001$) and age 18 years (OR, 2.01; 95% CI, 1.09-3.68; $P = .01$) in *FLG*-LOF variant subjects. 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; $P = .003$). FAS at age 10 years was associated with FA at age 18 years (OR, 4.88; 95% CI, 1.68-14.10; $P = .001$).

Indirect effect. Early eczema in subjects with a *FLG*-LOF variant had an indirect effect on FAS and FA (Table II). There was an indirect effect of *FLG*-LOF mutations through eczema at 1 to 2 years of age on FA at 1 and 2 years of age (OR, 2.81; 95% CI, 1.15-6.86; $P = .02$), FA at 4 years of age (OR, 15.48; 95% CI, 1.32 to >100; $P = .02$), and FAS at 4 years of age (OR, 2.18; 95% CI, 0.99-4.76; $P = .05$). Furthermore, *FLG*-LOF variants had an indirect effect on FA at age 10 years (OR, 10.0; 95% CI, 1.14-87.0; $P = .03$) through the occurrence of eczema at 1 to 2 years of age and FA at age 4 years. *FLG*-LOF mutation had an indirect effect through eczema at 4 years of age on FAS at 10 years of age (OR, 4.49; 95% CI, 1.44-13.99; $P = .01$) and 18 years of age (OR, 2.38; 95% CI, 1.19-4.74; $P = .01$) and FA at 18 years of age (OR, 21.93; 95% CI, 1.50 to >100; $P = .02$).

DISCUSSION

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

Filaggrin and FA

This is the first study to associate *FLG*-LOF mutations with all causes of FA rather than with a specific food allergen. Brown et al[16] described an association of *FLG*-LOF mutations and peanut allergy in 3 different populations. Their study supports a relationship of *FLG*-LOF mutations with peanut FA but does not consider other types of FA. Our study demonstrated a significant association (total effect by using path analysis) between FA and *FLG*-LOF mutations in older children and young adults (ie, 10 and 18 years old) but not during the earlier years. FAs in early childhood are often due to egg and milk allergy, which tend to improve, although in older children and young adults, peanut and seafood allergies become more prevalent and tend to persist (see Table E2 in this article's Online Repository at www.jacionline.org). Therefore our findings further confirm the association of *FLG*-LOF mutations with more persistent forms of FA.

FLG-LOF mutation pathway analysis

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

TABLE I. Prevalence of FA, FAS, and eczema in the study population over 18 years

Age	FA based on study criteria (% [95% CI])	Prevalence of food allergen sensitization (based on SPT response; % [95% CI])	Prevalence of eczema (% [95% CI])
1 y	5.3% (4.2% to 6.7%)	†	11.7% (9.9% to 13.5%)
2 y	4.4% (3.4% to 5.7%)	†	19.0% (16.7% to 21.2%)
4 y	5.0% (3.9% to 6.4%)	3.2% (2.1% to 4.3%)	12.1% (10.3% to 13.9%)
10 y	2.3% (1.7% to 3.3%),* $P < .001$, significant decrease in prevalence	4.5% (3% to 5.4%)	13.7% (11.9% to 15.5%)
18 y	4.1% (3.2% to 5.4%),* $P = .024$, significant increase in prevalence	21.4% (18.6% to 24.2%),* $P < .001$, significant increase in sensitization	12.1% (10.3% to 13.9%)

*Significant.
†SPTs at 1 and 2 years of age were limited to symptomatic children.

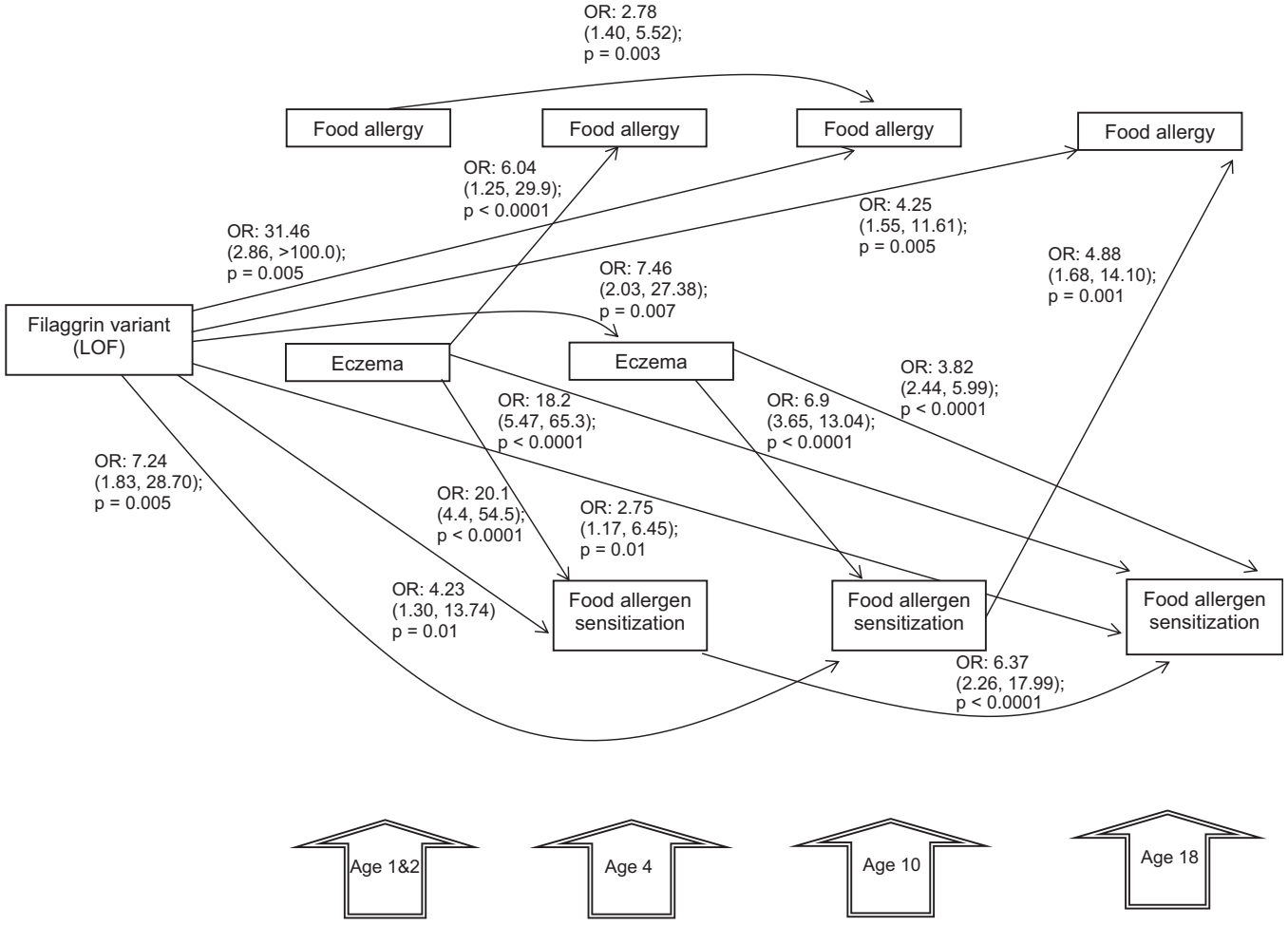

FIG 2. Analytic path model exploring the total effects of *FLG*-LOF mutation and eczema at ages 1 and 2 years and 4 years on FA and FAS at 4, 10, and 18 years of age. The path coefficient (total effects) represents the ORs. Goodness of fit adjusted for degrees of freedom = 0.99; comparative fit index = 0.99; root mean square error of approximation = ≤0.06; deviation between covariance structure and empiric covariance = $\chi^2/df \leq 2$; $P < .05$. Only significant direct paths are shown. The relationship between *FLG*-LOF mutations and FAS was not explored at 1 and 2 years of age because only symptomatic children underwent SPTs and FAS were data not available in the whole cohort.

relationship between *FLG*-LOF mutations and FA and are in keeping with current hypotheses on causality.[7] This suggests that 2 different mechanistic pathways might be sequentially involved in the pathogenesis of FA: a barrier dysfunction caused by *FLG*-LOF mutations and a barrier defect with associated inflammation caused by eczema leading to subsequent sensitization and immune response, all increasing the odds of having FA at different time points (Fig 3).

These results suggest a role for *FLG*-LOF mutations in FA development. The barrier defect associated with *FLG*-deficient subjects makes them susceptible to the development of cutaneous sensitization through antigen-presenting cells and systemic atopic

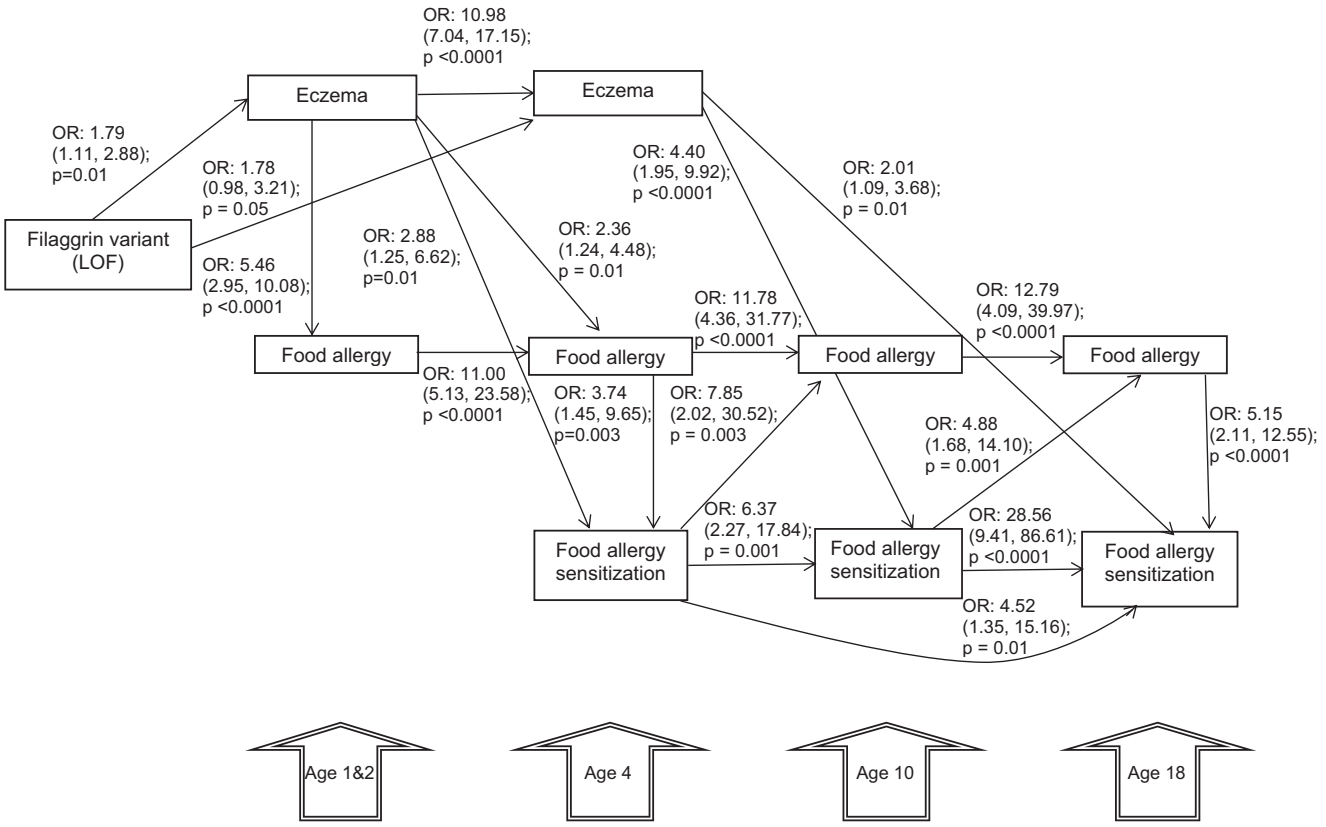

FIG 3. Analytic path model exploring the direct effects of *FLG*-LOF mutation and eczema at ages 1 and 2 years and 4 years on FA and FAS at 4, 10, and 18 years of age. The path coefficient (direct effects) represents the ORs. Goodness of fit adjusted for $df = 0.99$; comparative fit index = 0.99; root mean square error of approximation = ≤0.06; deviation between covariance structure and the empiric covariance = $\chi^2/df \leq 2$; $P < .05$. Only significant direct paths are shown. The relationship between *FLG*-LOF mutations and FAS was not explored at 1 and 2 years of age because only symptomatic children underwent SPTs and FAS data were not available in the whole cohort.

TABLE II. Significant results from indirect pathway analysis

FLG status	1 and 2 y	4 y	10 y	18 y	OR* (95% CI)	*P* value
FLG-LOF →	Eczema → FA†				2.81 (1.15 to 6.86)	.02
FLG-LOF →	Eczema →	FA†			15.48 (1.32 to >100)	.02
FLG-LOF →	Eczema →	Food sensitization†			2.18 (0.99 to 4.76)	.05
FLG-LOF →	Eczema →	FA →	FA†		10.0 (1.14 to 87.0)	.03
FLG-LOF →	→	Eczema →	Food sensitization†		4.49 (1.44 to 13.99)	.01
FLG-LOF →	→	Eczema →	→	Food sensitization†	2.38 (1.19 to 4.74)	.01
FLG-LOF →	→	Eczema →	Food sensitization →	FA†	21.9 (1.50 to >100.0)	.02

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

*OR represents the indirect association of *FLG*-LOF mutation on FA and food sensitization at different ages.

†The outcomes of the indirect paths.

response.[27-29] Peanut sensitization can occur through the topical application of peanut oil to the skin[8] and cutaneous exposure through the presence of peanut allergen in the environment,[30] and this process might 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 through 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 the presence of both inflammation and a defective barrier in patients with eczema lead to 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 allergens and aeroallergens) 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 through eczema in *FLG*-LOF mutations. *FLG*-LOF mutation has an indirect effect on FAS and FA in later childhood through the occurrence of eczema in earlier years, and this needs to be further explored by other longitudinal studies.

Flohr et al[32] examined the relationship between *FLG*-LOF mutations, atopic dermatitis, transepidermal water loss, and food-induced allergic sensitization in infancy. Their study found that children with eczema are more likely to be sensitized to food allergens independent of *FLG*-LOF status, severity of eczema, and transepidermal water loss. They did not show a relationship between *FLG*-LOF mutations and food sensitization, but instead, the severity of eczema was associated with FAS. Our study also showed a strong association between eczema (1 and 2 years and 4 years of age) and FAS at 4, 10, and 18 years of age. The association between *FLG*-LOF mutations and FA (total effects model) only becomes apparent in later childhood and adolescence. The effect of *FLG*-LOF mutations might be important in the maintenance of FA but could play less of a role in FA or FAS events in infancy, where atopic dermatitis might adversely affect the skin barrier and contribute to allergic sensitization and later development of FA in *FLG*-deficient children.

In summary, *FLG*-LOF mutations have an indirect effect on FA through eczema and FAS in the pathway. Our findings have discerned a relationship between *FLG*-LOF mutations, eczema, FAS, and FA and 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 in 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% to 94.3%), which ruled out a major bias because of loss to follow-up. The prevalence of eczema and allergic sensitization did not differ between the genotyped and full cohort data, supporting the generalizability of the study findings. A unique aspect of our study is the repeated longitudinal assessment of subjects throughout childhood, where each child acts as his or her own control subject.

The IOW birth cohort is dynamic; some children did not participate at 1 time point but rejoined at another. For this reason, comparisons were made between 2 time points, where FA information on a subject was available at both time points (1-2, 2-4, 4-10, and 10-18 years of age). We further explored this in a stable cohort in which FA data were available at all 5 time points (stable cohort, see Table E3 in this article's Online Repository at www.jacionline.org), 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 the 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 responses was performed at key stages, allowing FAS to be treated as a time-dependant covariate. The proportions of children who underwent SPTs were 67.4%, 71.1%, and 58.5% at 4, 10, and 18 years of age, respectively, which were much lower than the proportions of children followed up at the same time periods. This reduced the availability of food sensitization data. We also only had information on SPTs in symptomatic children at age 1 and 2 years, which limited the assessment of the relationship between allergic sensitization and FA in the early stages of childhood.

Our study is the first to investigate the relationship between *FLG*-LOF mutations, FAS, and FA longitudinally over 18 years (5 examinations), with each child acting as his or her own control subject 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 might be associated with more persistent forms of FA in older children and young adults. We further explored this association through path analysis by investigating effects of eczema and FAS in this pathway. *FLG*-LOF mutations had an indirect effect on FA in childhood through the occurrence of eczema and FAS in earlier years.

Impaired skin barrier function as a result of both eczema and *FLG*-LOF mutation seems to be a crucial common factor in the pathogenesis of FA, and improvement in the barrier function of skin in early childhood might 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.

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

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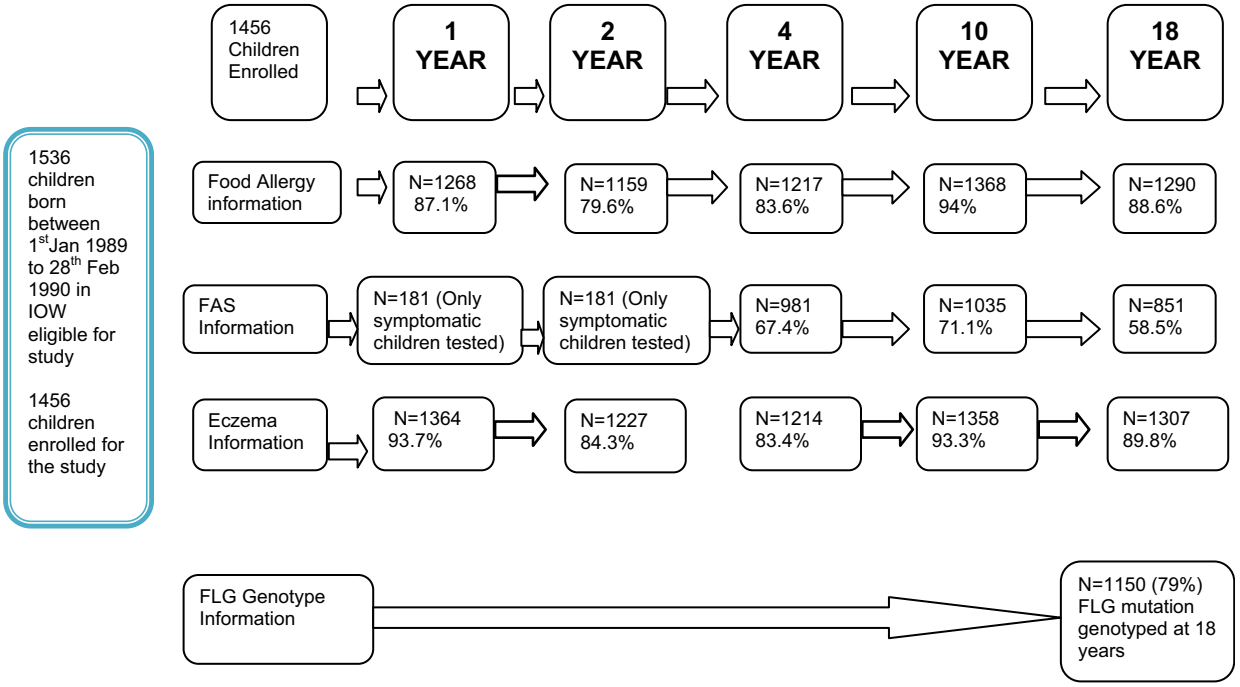

FIG E1. Participation data/availability of information at ages 1, 2, 4, 10, and 18 years in the IOW birth cohort study.

TABLE E1. Comparison of characteristics of the cohort and the genotyped subpopulation of the IOW birth cohort

Characteristics	Total population, no./total (%)	*FLG*-genotyped population,* no./total (%)
Sex		
Male	786/1536 (51.2)	569/1150 (49.5)
Female	750/1536 (48.8)	581/1150 (50.5)
Diagnosis of eczema		
1 y	182/1364 (13.3)	145/1065 (13.6)
2 y	259/1227 (21.1)	207/978 (21.2)
4 y	147/1214 (12.1)	121/1008 (12.0)
10 y	186/1358 (13.7)	164/1117 (14.6)
18 y	161/1307 (12.3)	132/1086 (12.2)
Diagnosis of FA		
1 y	67/1268 (5.3)	58/998 (5.8)
2 y	51/1157 (4.4)	45/935 (4.8)
4 y	61/1217 (5.0)	56/1010 (5.5)
10 y	32/1368 (2.3)	29/1123 (2.6)
18 y	51/1290 (4.0)	40/1074 (3.8)

*No significant differences were found between the total population and the *FLG*-genotyped subgroup. The genotyped population represents a subgroup of children in whom genotype was determined for the 5 mutations.

TABLE E2. Prevalence of food allergies based on food groups and age

Food group	1 y (n = 1268)	2 y (n = 1159)	4 y (n = 1217)	10 y (n = 1368)	18 y (n = 1290)
All FA	67 (5.3%)	51 (4.4%)	61 (5.0%)	32 (2.3%)	52 (4.0%)
Cow's milk	44 (3.5%)	19 (1.6%)	32 (2.6%)	7 (0.5%)	4 (0.3%)
Hen's egg	14 (1.1%)	15 (1.3%)	17 (1.4%)	8 (0.6%)	4 (0.3%)
Wheat	6 (0.5%)	5 (0.4%)	3 (0.3%)	1 (0.1%)	6 (0.5%)
Soya	—	—	4 (0.3%)	—	—
Fish	2 (0.2%)	—	2 (0.2%)	2 (0.2%)	2 (0.2%)
Shellfish	—	—	—	2 (0.2%)	3 (0.2%)
Peanut	1 (0.08%)	2 (0.2%)	4 (0.3%)	6 (0.4%)	13 (1%)
Tree nuts	—	1 (0.1%)	2 (0.2%)	2 (0.2%)	7 (0.5%)
Fruits	10 (0.8%)	11 (0.9%)	9 (0.7%)	4 (0.3%)	11 (0.9%)
Kiwi	—	—	—	1 (0.1%)	3 (0.2%)
Vegetables	2 (tomatoes [0.2%])	3 (tomatoes [0.3%])	4 (tomatoes/Brussels sprouts [0.3%])	4 (tomatoes [0.3%])	5 (tomatoes/leek/ cucumber [0.4%])
Others	—	2 (0.2%)	2 (0.2%)	2 (0.2%)	3 (0.3%)

FA based on study criteria is defined as follows: reaction to known food allergen, typical IgE reaction symptoms, and reaction timing of less than 4 hours.

TABLE E3. FA prevalence in the whole population and the stable cohort

Age group	Population with food allergy (whole cohort)	Percent (95% CI)	Population with food allergy (stable cohort)	Percent (95% CI)
1 y	67/1268	5.3 (4.2-6.7)	44/843	5.2 (3.9-6.9)*
2 y	51/1159	4.4 (3.4-5.7)	33/843	3.9 (2.8-5.4)*
4 y	61/1217	5.0 (3.9-6.4)	39/843	4.6 (3.4-6.3)*
10 y	32/1368	2.3 (1.7-3.3)	18/843	2.1 (1.4-3.3)*
18 y	52/1290	4.1 (3.2-5.4)	30/843	3.6 (2.5-5)*

*No significant statistical difference in the prevalence of FA between the whole population and stable cohort.