Food allergy and gastrointestinal disease

Filaggrin loss-of-function mutations are associated with persistence of egg and milk allergy

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GRAPHICAL ABSTRACT

Background: A genetic defect in the epidermal barrier protein filaggrin (FLG) plays a major role in the etiology of eczema and associated allergic airways diseases. However, it is still controversial to what extent loss-of-function (LOF) mutations in FLG contribute to the development and persistence of food allergies.

Objectives: This study tested association of FLG LOF mutations with allergic reactions to diverse foods and investigated their potential effect on the persistence of early food allergies.

Methods: This study recruited 890 children with challenge-proven food allergy for the German Genetics of Food Allergy Study (GOFA). Longitudinal data were available for 684 children. All children were clinically characterized, including their allergic responses to specific foods, and genotyped for the 4 most common LOF mutations in FLG; R501X, 2282del4, R2447X, and S3247X. Associations between FLG mutations and food allergies were analyzed by logistic regression using the

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German Multicenter Allergy Study cohort as the control population.
Results: FLG mutations were associated with allergies to diverse foods including hen’s egg (HE), cow’s milk (CM), peanut, hazelnut, fish, soy, cashew, walnut, and sesame with similar risk estimates. Effects remained significant after adjusting for the eczema status. Interestingly, FLG mutations increased the risk of a persistent course of HE and CM allergy.
Conclusions: Using the gold standard for food allergy diagnosis, this study demonstrates that FLG LOF mutations confer a risk of any food allergy independent of eczema. These mutations predispose to the persistence of HE and CM allergy and should be considered in the assessment of tolerance development. (J Allergy Clin Immunol 2022;150:1125-34.)

Key words: Persistence, food allergy, hen’s egg, cow’s milk, FLG, loss-of-function mutations, Genetics of Food Allergy Study, GOFA, double-blind placebo-controlled food challenge, eczema

Food allergy (FA) is a global health problem with increasing prevalence over the past decades; in developed countries up to 10% of children are affected.1-3 FA can trigger severe allergic responses and is the leading cause of life-threatening anaphylaxis in childhood.4,6 The main elicitors in infancy are hen’s egg (HE) and cow’s milk (CM), whereas allergic responses to peanut and tree nuts usually occur later in childhood.7 Therapeutic options for FA are limited. Avoidance of the allergenic food is usually the method of choice. While a large proportion of HE and CM allergy resolves spontaneously in early childhood,8,9 some patients develop persistent FA with severe impact on their quality of life. The identification of patients at risk of persistent FA as well as understanding the underlying molecular mechanisms are therefore of major importance for the development of novel therapeutic strategies.

FLG is an epidermal protein that is essential for the integrity of the skin barrier. FLG monomers, which are cleaved from the large precursor protein profilaggrin, form tight bundles with keratin intermediate filaments and are linked to the protein-lipid cornified cell envelope.10 This complex structure provides physical strength and the main barrier function of the stratum corneum. In addition, FLG degradation products serve as natural moisturizing factor, which is essential for elasticity of the stratum corneum and for normal desquamation.10 Accordingly, defective FLG was linked to an impaired skin barrier and to a loss of natural moisturizing factor.11 Loss-of-function (LOF) mutations in FLG were identified as the main genetic risk factors for eczema.12 Interestingly, FLG mutations are also involved in the development of eczema-associated allergic airway diseases such as asthma and allergic rhinitis,13,14 pointing to a key function in the pathogenesis of allergies. FLG is specifically expressed in stratified epithelia, mostly in the upper layers of the epidermis, but also in oral epithelia and esophageal mucosa.15,16 In a mouse model, enhanced penetration of allergens through an impaired skin barrier led to systemic sensitization and subsequent allergic reactions on allergen contact in distant organs, demonstrating a link between epidermal barrier defect and airway diseases.17

FLG LOF mutations were detected in all ethnic groups with similar cumulative allele frequencies of about 5%, but mutation patterns were population-specific.18 In populations of European ancestry, only 4 LOF mutations with allele frequencies >0.1% were identified: p.Arg501Ter, p.Ser761CysfsTer36, p.Arg2447Ter, and p.Ser3247Ter.19 Each of the FLG mutations have an equivalent molecular biological effect, leading to truncated profilaggrin and complete absence of functional FLG monomers.19,20 Because the mutations are located on different haplotypes, they are usually analyzed in a combined manner yielding the FLG genotypes wild type (wt/wt), heterozygous (wt/mut), and homozygous mutation (mut/mut) including homozygous and compound heterozygous mutation carriers.19

While the associations of FLG LOF mutations with eczema, asthma, and allergic rhinitis are well established, the role of FLG in FA is less clear. Genetic studies on FA often suffer from small sample sizes and weak phenotype definitions due to a lower prevalence and a higher diagnostic effort. To date, only a few studies on FLG in FA were conducted with different study designs and outcomes. In the Australian HealthNuts study, FLG LOF mutations increased the risk of food sensitization at age 1 year, but without an additional effect on FA.21 In a Swedish birth cohort, FLG LOF mutations were associated with peanut sensitization only at 4 years of age.22 In adults of a Danish cross-sectional study, a FLG effect on food sensitization was demonstrated only in the presence of eczema.23 In addition, the English Isle of Wight (IoW) birth cohort reported no direct effect of FLG LOF mutations on FA, but did report an indirect effect through eczema and food sensitization in early childhood.24 Finally, associations of FLG LOF mutations with peanut allergy and with clinical reactivity to food after adjusting for eczema were identified in 2 case control studies from England and the Netherlands, respectively.25,26 However, these results were difficult to assess due to the different age groups under study (from infants to adults), diverse phenotype definitions ranging from questionnaire-based to the recommended gold standard, double-blind, placebo-controlled food challenges (DBPCFCs),2,3 and the inclusion of varying allergenic foods across studies.

In this study, we investigate the role of FLG LOF mutations in the large Genetics Of Food Allergy Study (GOFA) including 890 children with FA who are of European ancestry from Germany. The majority of children was diagnosed by DBPCFC, the current gold standard. We demonstrate associations of FLG mutations with allergies to a wide range of allergenic foods, and we show that the FLG effect remains significant after adjusting for the eczema status. In addition, FLG mutations increase the risk of persistent HE and CM allergy, the 2 most common FAs in childhood.
METHODS

Study population

Children of the GOFA were recruited at pediatric clinics in Berlin, Wangen, Oldenburg, and Hannover. In line with the current guidelines, FA was diagnosed based on an oral food challenge (OFC) (n = 766), most of which (n = 658; 85.9%) were conducted in a double-blind, placebo-controlled setting. All children with a suspected FA based on current symptoms or a clinical history plus sensitization to the corresponding food (elevated specific IgE and/or positive skin prick test) underwent an OFC. Children with a convincing history of an immediate, allergic reaction plus specific sensitization to the same food (IgE > 0.35 kU/L) were included as cases without further challenge (n = 124), as OFC was contraindicated due to the risk of a severe allergic reaction. OFCs were performed in an inpatient hospital setting under physicians’ supervision. Children and their parents were instructed to quit any systemic anti-inflammation medication 1 week before OFC. Only 1 food or placebo was administered in 7 escalating doses at 30-minute intervals. The cumulative allergen dose was given the next day if the 7 doses were tolerated without reaction. Consistent with the Practical Allergy (PRACTALL) guidelines, food challenges were scored as positive if the 7 doses were tolerated without reaction. A physician’s diagnosis of eczema was made according to standard criteria including a chronic or chronically relapsing pruritic dermatitis with allergen-specific IgE levels determined using the ImmunoCAP test (Thermo Fisher Scientific/Phadia, Uppsala, Sweden). A pediatrician’s diagnosis of eczema was made according to standard criteria in the presence of a chronic or chronically relapsing pruritic dermatitis with the typical morphology and distribution.5,26 In total, 890 children with FA for whom DNA samples were available for genotyping were included in this study. All individuals were of European ancestry as previously confirmed by principal component analysis.3,25

Children of the German Multicenter Allergy Study (MAS) were used as the control population. This cohort has previously been described in detail.22,23 MAS consists of 1314 children born in 1990. Children were followed at the age of 1, 3, 6, 12, 18, and 24 months, and at yearly intervals thereafter until the age of 13 years. They were extensively characterized regarding their eczema and asthma status. However, food challenges were not performed and robust data on FA were not available. Accordingly, children from MAS were used as population-based controls. For regression analyses, eczema was defined by the presence of (1) a reported physician’s diagnosis, (2) a parental report of eczema symptoms, or (3) visible eczema at the time of follow-up. DNA samples were available from 871 children of German descent. All samples were genotyped for the 4 FLG LOF mutations indicated below. The institutional review boards of all centers approved the study, and written informed consent was obtained from all participants or their legal guardians.

Genotyping

Genomic DNA was isolated from whole blood by standard methods. In all individuals, the most prevalent FLG LOF mutations in Europeans (R501X, 2282del4, R2447X, and S3247X) were genotyped by using TaqMan allelic discrimination, fluorescence-based semi-automated allele-sizing technology, or restriction enzyme digestion, as described previously.3,13,19

Statistical analyses

Because all investigated FLG mutations are LOF mutations located on different haplotypes, we combined them for the analysis as FLG status wt/wt, wt/mut, or mut/mut where wt/mut indicates a carrier of any 1 LOF mutation and mut/mut indicates a carrier of 2 mutated alleles (ie, homozygous or compound heterozygous). The association of the combined FLG mutations with FA was tested under an additive model with PLINK14 using a logistic regression model adjusted by sex. Afterward, models were rerun including also eczema status as covariate to assess whether significant associations were dependent or independent of an eczema diagnosis. Association of the combined FLG mutations with age at FA diagnosis was analyzed by linear regression models including sex and eczema as covariates.3,25 To assess the effect of FLG LOF mutations on the persistence of FA, we performed a Kaplan-Meier survival analysis.15 Data were preprocessed so that for each individual with allergy, the data contained information about a new “event” happening as either “1” if participant had achieved tolerance, or “0” if participant was censored (or study termination), at each time point assessed. Kaplan-Meier curves enable analysis of incomplete sets of data (ie, after participants are lost to follow-up from visit to visit). Participants were stratified by FLG mutations carrier status (carriers vs noncarriers). Survival objects and Kaplan-Meier curves were fitted using the package “survival” in R (R Foundation, Vienna, Austria). Kaplan-Meier curves were visualized using the packages “survminer” and “ggpubr.” Log-rank P values were calculated from the “survival” package and visualized using “survminer.” Specific IgE levels were categorized into CAP classes 1 to 6. Correlation between allergen-specific IgE determined at the first visit below 2 years of age and FA persistence was assessed using the Kruskal-Wallace rank sum test. Finally, risk factors for persistence were analyzed using a 2 × 2 contingency table and Pearson chi-square test; for the FLG LOF mutations an allele test was performed.

RESULTS

Characteristics of the study population: allergies to specific foods, comorbidities, FLG LOF mutations

A detailed description of the study population is provided in Table I. The most common FAs were those to HE (55.6%), peanut (39.1%), and CM (30.7%). Allergic symptoms on food challenge mainly involved the skin (93.6%), the gastrointestinal tract (32.2%), and the respiratory tract (23.2%). In 53.1% of children, the allergic response affected 2 or more organ systems (Table I). For 684 cases, at least 1 follow-up visit was available. The mean follow-up period was 39 months.

Overall, allergic comorbidities were present in 91% of children, with eczema being the most common (82.9%) among all children with FAs (Table I). The pattern of comorbidities varied according to the age of the children. In early childhood, the majority of children with FAs had concomitant eczema; below 4 years of age, asthma and hay fever were diagnosed only in 6.7% and 6.9% of the patients, respectively (see Fig E1 in this article’s Online Repository at www.jacionline.org). Higher rates of asthma (37.4%) and hay fever (42.3%) were found in children who were followed until school age (6-8 years). Finally, of the children with FAs who had their last examination at ≥8 years of age, 63.7% had developed asthma and 54.8% hay fever.

All children were genotyped for the 4 most common FLG LOF mutations present in European populations: p.Arg501Ter, p.Ser766CysfsTer36, p.Arg2447Ter, and p.Ser3247Ter. In children with FAs, the allele frequency of the 4 FLG mutations combined was 13.3%, yielding a carrier frequency of 23.7% (Table I).

FLG mutations increase the risk of FA independent of the allergenic food

All 4 mutations under study were more frequent among food allergic cases with similar risk estimates (see Table E1 in this article’s Online Repository at www.jacionline.org). Because they are located on different haplotypes, we performed all analyses using the combined genotype of the 4 mutations. Associations of the FLG mutations with allergies to different allergenic foods was analyzed by logistic regression using children of the German MAS study as population-based controls (n = 871). We found a strong association between the FLG status (odds ratio [OR] = 2.80; P = 4.4 × 10⁻¹⁵) and FA (Table II). This effect was not
TABLE I. Characteristics of the GOFA study

| GOFA (N = 890) | Male sex | 573 (64.4) |
| GLF mutation carrier | 211 (23.7) |
| Age at first diagnosis, mean ± SD | 2.36 ± 2.69 |
| Allergy to | Hen’s egg | 495 (55.6) |
| Cow’s milk | 273 (30.7) |
| Peanut | 348 (39.1) |
| Hazelnut | 145 (16.3) |
| Other foods| 225 (25.3) |
| Polyvalent FA | 405 (45.5) |
| Reacting organ system (at first challenge) | n = 715 |
| Skin—eczema | 50 (7.0) |
| Skin—other symptoms | 619 (86.6) |
| Gastrointestinal tract | 230 (32.2) |
| Lower respiratory tract | 166 (23.2) |
| Nasal mucosa/eye conjunctiva | 122 (17.1) |
| Nervous system | 58 (8.1) |
| Cardiovascular system | 16 (2.2) |
| ≥2 Organ systems affected | 380 (53.1) |
| Allergic comorbidities | n = 890 |
| Eczema | 738 (82.9) |
| Asthma | 201 (22.6) |
| Hay fever | 211 (23.7) |

Values are n (%) unless otherwise indicated.
*Other foods include wheat, fish, soy, cashew, walnut, pea, sesame, lens, and 26 additional foods with <1% of children in GOFA affected.
†Other skin symptoms are urticaria, angioedema, erythema, itch, flush, and wheals.

restricted to specific foods as similar effect sizes and significant P values (significance threshold corrected for the number of tests, P < .005) were observed for almost all foods tested. Only the association with wheat allergy comprising 55 cases did not reach significance. Because defective FLG is a major cause of eczema and because FA is often accompanied by eczema, we tested whether the observed association was due to eczema rather than FA. After adjusting for the eczema status, the effect of the FLG mutations on FA decreased slightly (OR_adj = 2.10; P = 1.4 × 10^-5) but remained significant indicating that FLG mutations confer a risk of FA independent of eczema. We additionally conducted an eczema-stratified analysis, to test whether the FLG effect was different in children with eczema and in children without eczema. In both subgroups, significant effects of similar size were observed demonstrating that the FLG effect on FA was independent of the presence of eczema (see Table E2 in this article’s Online Repository at www.jacionline.org). GOFA also enabled us also to investigate the effect of FLG mutations on polyvalent FA as 45% of the children had developed allergic responses to 2 or more foods. FLG mutations did not increase the risk of polyvalent versus monovalent FA (P = .18).

**FLG LOF mutations are not associated with organ-specific allergic responses**

To test the FLG status for association with organ-specific responses, we used data from the first OFC of each study participant (Fig 1). In carriers of FLG LOF mutations, there was a trend that skin and nasal mucosa/eye conjunctiva were less frequently involved in allergic reactions. However after correcting for the number of organ systems tested (n = 7), none of the association P values reached the significance threshold of P < .0071.

**Association between FLG LOF mutations and the age at FA diagnosis**

An age-of-onset analysis was performed for the 4 most common allergenic foods in GOFA: HE, peanut, CM, and hazelnut. According to the introduction of a specific food to the children’s diet, allergies to CM and HE were usually diagnosed within the first 2 years of life (mean age of 1.4 years and 1.8 years, respectively), whereas hazelnut or peanut challenges were usually performed later (mean age of 3.3 years and 3.6 years, respectively). Comparing the age at FA diagnosis in FLG LOF mutation carriers versus noncarriers in a linear regression model revealed no difference for any of the foods (Table III). Because patients with FAs were asked to return for yearly follow-up visits to evaluate them for disease persistency or resolution, we also analyzed the age at the last positive OFC. Here we identified a significant difference in patients with allergies to CM (P = 1.1 × 10^-4) and a nominally significant effect for HE (P = .039) (Table III). Carriers of FLG LOF mutations presented at the clinics for a longer period than those who did not carry a mutation, suggesting an effect of the combined FLG mutations on the persistency of CM and HE allergy.

**Effect of FLG LOF mutations on the persistence of HE and CM allergy**

In contrast to peanut and tree nut allergy, FA against HE or CM tends to resolve spontaneously in early childhood. To investigate the effect of FLG on the persistence of HE and CM allergy in more detail, we performed a survival analysis in carriers of the FLG mutations versus noncarriers (Fig 2). Resolution of the respective allergy was used as the end point, which was either determined by a negative food challenge or by consumption of the formerly allergenic food without symptoms. We found significant differences in the curves for both HE and CM allergy (P = .032 and P < .0001, respectively). Carriers of the FLG LOF mutations were more likely to experience a persistent disease course than noncarriers were. The main separation of the curves occurred between 2 and 4 years, with minor increase of the deviation thereafter.

According to the results of the survival analysis, we defined 2 groups of children; those whose allergy resolved early (negative result for HE or CM at the first rechallenge between 2 and 4 years) and those whose allergy persisted (positive OFC for HE or CM, respectively, >2 years). Of 495 and 273 children with an early diagnosis of HE and CM allergy, respectively, 341 and 177 children fulfilled these criteria and were included in the analysis (Table IV). HE allergy resolved in 61 children (17.9%) at the first rechallenge (at a mean age of 35 months), CM allergy resolved in 70 children (39.5%) at a mean age of 31 months. Among children with persistent allergy, the mean age at the last follow-up visit with a positive OFC was 63 months for HE allergy (n = 280) and 62 months for CM allergy (n = 107).

Comparing the frequencies of carriers of at least 1 FLG LOF mutation between children with transient and persistent HE and CM allergy, respectively, yielded a significant increase of FLG mutation carriers among children whose allergy did not resolve early (Table IV). Among children who were HE-allergic with a positive rechallenge, the allele frequency of the combined FLG mutations was 16.8% compared with 6.6% in children whose HE allergy resolved. The presence of a FLG LOF mutation significantly increased the risk to develop persistent HE allergy (OR =
2.87; P = .004). A similar effect was observed for CM allergy. Here the allele frequency increased from 8.6% in children with a negative rechallenge to 23.8% in children with a positive rechallenge (OR = 3.34, P = .0002). Overall, 29.6% and 39.3% of the children with persistent HE and CM allergy, respectively, were carriers of an FLG LOF mutation. Notably, the presence of eczema or additional FAs had no impact on the persistence of HE or CM allergy.

### Association of FLG mutations with organ-specific allergic responses

![Graph showing association of FLG LOF mutations with organ-specific allergic responses.](https://www.jacionline.org)

The significance threshold was set at P < .0071 according to the number of organ systems tested (n = 7). *Other skin symptoms are urticaria, angioedema, erythema, itch, flush, wheals.

### Table II. Association of FLG mutations with food allergic phenotypes

| Allergic to     | n_cases | n_controls* | OR_unadj | 95% CI_unadj | P_value_unadj | OR_adj | 95% CI_adj | P_value_adj |
|----------------|---------|-------------|----------|--------------|---------------|--------|------------|-------------|
| Any food       | 890     | 871         | 2.80     | (2.16-3.62)  | 4.4E−15       | 2.10   | (1.59-2.77) | 1.4E−07     |
| HE             | 495     | 871         | 2.85     | (2.14-3.79)  | 6.0E−13       | 1.81   | (1.33-2.46) | 1.8E−04     |
| Peanut         | 348     | 871         | 3.05     | (2.24-4.14)  | 1.0E−12       | 2.43   | (1.76-3.36) | 8.0E−08     |
| CM             | 273     | 871         | 2.86     | (2.07-3.94)  | 1.7E−10       | 1.86   | (1.32-2.62) | 3.7E−04     |
| Hazelnut       | 145     | 871         | 2.79     | (1.89-4.12)  | 2.4E−07       | 1.85   | (1.23-2.78) | 3.3E−03     |
| Wheat          | 55      | 871         | 1.82     | (0.94-3.50)  | .075          | 1.11   | (0.57-2.19) | .75         |
| Fish           | 42      | 871         | 3.60     | (2.02-6.44)  | 1.5E−05       | 2.25   | (1.24-4.07) | 7.4E−03     |
| Soy            | 37      | 871         | 2.80     | (1.41-5.54)  | 3.2E−03       | 1.96   | (0.97-3.94) | .06         |
| Cashew         | 31      | 871         | 3.17     | (1.52-6.61)  | 2.1E−03       | 2.21   | (1.04-4.69) | .039        |
| Walnut         | 25      | 871         | 3.50     | (1.62-7.55)  | 1.4E−03       | 2.27   | (1.04-4.95) | .04         |
| Sesame         | 16      | 871         | 3.89     | (1.62-9.36)  | 2.4E−03       | 2.71   | (1.11-6.58) | .028        |
| Polyvalent FA  | 405     | 485         | 1.20     | (0.92-1.56)  | .18           | 1.09   | (0.84-1.43) | .52         |

* Controls are from the German Multicenter Allergy Study (MAS) except for “Polyvalent FA,” which compares only cases with polyvalent FA versus monovalent FA.

† The significance threshold was set at P < .005 according to the number of food allergens tested (n = 10).

### The role of allergen-specific IgE levels in the persistence of HE or CM allergy

We additionally investigated whether the levels of specific IgE to HE or CM at the first visit correlated with the persistence of the respective FA. Specific IgE levels were categorized into 6 CAP classes. For HE, we detected a significant association of higher HE-specific IgE with persistence (median CAP_persistence = 3 vs median CAP_nonpersistence = 2; P_HE = 1.5 × 10^{-4} (Fig 3, A). For CM, a similar effect was observed (median CAP_persistence = 3 vs median CAP_nonpersistence = 2; P_CM = 1.3 × 10^{-5} (Fig 3, B). On the other hand, there was no difference in the IgE levels between carriers and noncarriers of FLG mutations, indicating that elevated specific IgE and FLG LOF mutations are independent risk factors for the persistence of HE and CM allergy (see Fig E2 in this article’s Online Repository at www.jacionline.org).

### Association of FLG LOF mutations with the severity of the allergic response

To assess whether FLG LOF mutations had an effect on the severity of the allergic response to HE or CM, we used data from the first OFC with the respective food. We applied the World Allergy Organization grading system to classify the allergic responses into 5 categories from mild symptoms involving only 1 organ (grade 1) to severe lower/upper airway or cardiovascular reactions.
with peanut allergy and others considered FAs against diverse foods jointly to get sufficient numbers of cases. In CM allergy (eczema effect), we demonstrate that LOF mutations in FLG predispose to allergy against a wide range of foods, an effect independent of the known eczema effect. We additionally show that FLG LOF mutations increase the risk of a persistent course of HE or CM allergy suggesting their use as potential prognostic markers.

Previous studies investigated the association of FLG mutations with peanut allergy and others considered FAs against diverse foods jointly to get sufficient numbers of cases. In GOFA, we recruited a large number of children with FAs, almost doubling the number of cases compared with those of previous reports. This allowed us, together with a well-defined phenotype mainly based on DBPCFCs, to perform allergen-specific analyses. We identified significant associations between FLG mutations and almost all FAs tested including HE, CM, peanut, hazelnut, fish, soy, cashew, walnut, and sesame. Only the association with wheat allergy did not reach significance, which could be due to the lower numbers of cases. Considering the known association of FLG mutations with eczema and its high prevalence among children from GOFA (>80%), we investigated the association with wheat allergy (PHE = .60 and PCM = .69) (see Table E3 in this article’s Online Repository at www.jacionline.org).

**DISCUSSION**

Markers for progression of early FA are urgently needed. Here we demonstrate that LOF mutations in FLG predispose to allergy against a wide range of foods, an effect independent of the known eczema effect. We additionally show that FLG LOF mutations increase the risk of a persistent course of HE or CM allergy suggesting their use as potential prognostic markers.

**TABLE III.** Effect of the FLG mutations on the age at diagnosis of FA

| Allergic to          | All                  | FLGmut carriers | FLGwt carriers | Beta  | SE    | P value* |
|----------------------|----------------------|-----------------|----------------|-------|-------|----------|
| Any food, n          | 888                  | 211             | 677            |       |       |          |
| Age at first diagnosis| 2.36 ± 2.69          | 2.37 ± 2.64     | 2.35 ± 2.71    | 0.124 | 0.172 | .47      |
| Age at last diagnosis | 4.40 ± 3.61          | 4.68 ± 3.49     | 4.32 ± 3.64    | 0.391 | 0.236 | .098     |
| HE, n                | 495                  | 122             | 373            |       |       |          |
| Age at first diagnosis| 1.78 ± 1.91          | 1.84 ± 2.05     | 1.77 ± 1.87    | 0.160 | 0.167 | .34      |
| Age at last diagnosis | 3.18 ± 2.92          | 3.61 ± 3.18     | 3.04 ± 2.83    | 0.532 | 0.257 | .039     |
| CM, n                | 272                  | 65              | 207            |       |       |          |
| Age at first diagnosis| 1.38 ± 1.75          | 1.41 ± 1.39     | 1.38 ± 1.85    | 0.196 | 0.196 | .32      |
| Age at last diagnosis | 2.42 ± 2.67          | 3.43 ± 3.09     | 2.11 ± 2.45    | 1.156 | 0.294 | .00011   |
| Peanut, n            | 347                  | 89              | 258            |       |       |          |
| Age at first diagnosis| 3.64 ± 2.91          | 3.82 ± 3.05     | 3.59 ± 2.87    | 0.083 | 0.297 | .78      |
| Age at last diagnosis | 5.30 ± 3.68          | 5.28 ± 3.70     | 5.32 ± 3.68    | −0.2223 | 0.3717 | .55   |
| Hazelnut, n          | 145                  | 34              | 111            |       |       |          |
| Age at first diagnosis| 3.27 ± 2.43          | 3.40 ± 2.07     | 3.23 ± 2.53    | 0.397 | 0.382 | .3       |
| Age at last diagnosis | 4.40 ± 2.90          | 4.22 ± 2.45     | 4.45 ± 3.02    | 0.1118 | 0.457 | .81     |

Values are mean ± SD unless otherwise indicated.

*The significance threshold was set at P < .0003 according to the number of independent tests performed for the 4 allergens (n = 8).

**FIG 2.** Persistence rates in (A) HE allergy and (B) CM allergy are dependent on the FLG mutation carrier status. Using a Kaplan-Meier survival curve, the persistence of FA from 0 to >8 years was analyzed in carriers of the FLG LOF mutations (FLG) versus in noncarriers (WT). Resolution of the respective allergy was used as the end point. The number of individuals who were lost to follow-up before reaching the end point is indicated.
without eczema, supporting an eczema-independent effect of the epidermal barrier on FA. 38

The role of FLG mutations in FA has been controversially discussed and seems to be dependent on the age group analyzed. In the Australian HealthNuts study, eczema-adjusted results revealed an association between FLG mutations and increased food IgE levels, mainly to HE and CM among 1-year-old infants but an additional effect on FA was not observed.21 This outcome may be due to a lack of power to distinguish sensitization from clinically relevant FA, as the vast majority of children who were food sensitized were OFC positive (88%).

The IoW birth cohort study reported an association of FLG mutations with FA only in children from 10 years onward following food sensitization and eczema in early childhood.24 Though the number of food allergic cases was small with a maximum of 67 children with FAs at age 1 year, this study pointed to an effect

### TABLE IV. Risk factors for a positive follow-up challenge in children with early allergy to HE or CM

| Risk factors | HE allergy (n = 341) | CM allergy (n = 177) |
|--------------|----------------------|----------------------|
|              | Negative challenge/  | Positive challenge/   |
|              | consumption >2 years | reaction >2 years     |
|              | (n = 61)             | (n = 280)            |
| Ecema        | 56 (91.8)            | 254 (90.7)           | 0.87 (0.32-2.37) | .79 |
| FLG mutations| 8/0 (AF, 6.6)        | 72/11 (AF, 16.8)     | 2.87 (1.36-6.09) | .004 |
|              | 52 (74.3)            | 87 (81.3)            | 1.51 (0.73-3.10) | .27 |
|              | 63 (90.0)            | 95 (88.8)            | 0.88 (0.33-2.36) | .79 |
|              | 10/1 (AF, 8.6)       | 33/9 (AF, 23.8)      | 3.34 (1.71-6.52) | .0002 |
| Other FAs    | 34 (55.7)            | 183 (65.4)           | 1.50 (0.85-2.63) | .16 |
| Eczema       | 56 (91.8)            | 254 (90.7)           | 0.87 (0.32-2.37) | .79 |
| FLG mutations| 8/0 (AF, 6.6)        | 72/11 (AF, 16.8)     | 2.87 (1.36-6.09) | .004 |
|              | 52 (74.3)            | 87 (81.3)            | 1.51 (0.73-3.10) | .27 |
|              | 63 (90.0)            | 95 (88.8)            | 0.88 (0.33-2.36) | .79 |
|              | 10/1 (AF, 8.6)       | 33/9 (AF, 23.8)      | 3.34 (1.71-6.52) | .0002 |

Values are n (%) unless otherwise indicated.
AF, Allele frequency; het, heterozygous carriers; hom, homozygous carriers.
*The significance threshold was set at \( P < .0083 \) according to the number of independent tests performed (n = 6).
of the FLG LOF mutations on FA mainly in older children. Our study found an age-dependent effect of the FLG LOF mutations in children with early allergies to HE and CM. We found a strong association between FLG mutations and persistent allergy to HE and CM (OR_{HE} = 2.9 and OR_{CM} = 3.3). Survival curves indicated that in HE and CM allergy the main effect of FLG occurs much earlier than in the IoW study, between 2 and 4 years, at a time point when natural tolerance usually develops. Additionally, the IoW results may have been influenced by a less strict phenotype definition using questionnaire-reported FA symptoms, the inclusion of children with a negative skin prick test as well as by the lack of stratification for specific food allergens.

Therapeutic options for FA are still very limited. Apart from avoidance of the allergenic food, tolerance induction through oral immunotherapy (OIT), the repeated application of small allergen doses below the allergenic threshold level, is becoming more and more important. Recently, the first OIT product for the treatment of peanut allergy was approved. However, OIT is largely restricted to children with a severe and more likely persistent disease course; trials have therefore focused mainly on peanut allergy, which is most common among school children and adults. In contrast, the most prevalent FAs in infancy are those against HE or CM. Allergic responses to HE and CM typically develop during the first year of life and often show a transient disease course with most affected children acquiring clinical tolerance within a few years. However, some individuals develop a persistent FA. The factors influencing these divergent disease trajectories are largely unknown. It is therefore essential to gain a better understanding of the underlying mechanisms and to identify clinical features and biomarkers for tolerance development or persistence of FA. FLG LOF mutations are the first genetic marker associated with persistent HE or CM allergy. They might be used as prognostic markers to identify children who would most likely benefit from an OIT to HE and CM in the future. In addition, in carriers of a FLG mutation, the interval between oral rechallenges required to test for persistence of reactivity should be delayed, which would avoid undue stress in the patient and reduce the risk of severe reactions.

Other biomarkers associated with persistent HE and CM allergy were egg- and milk-specific IgE levels, respectively, as well as the diversity of epitope-specific IgEs. Confirming previous data, we found a highly significant association of allergen-specific IgE levels with the persistence of HE and CM allergy. HE and CM epitope-specific IgE levels were not available in GOFA. It would be interesting to test whether these phenotypes are also correlated with FLG mutations. In addition, a specific innate immune signature characterized by an increased number of monocytes and dendritic cells was associated with persistent HE allergy in the HealthNuts Study. Finally, the gut microbiome seems to play a role in FA resolution because its composition in early infancy differed significantly between transient and persistent CM allergy. A risk score combining genetic and immunological data could be a promising approach to identify children at risk of persistent HE and CM allergy.

A few limitations of our study have to be mentioned. While the definition of cases was based on the gold standard DBPCFC, controls were unscreened for the absence of FA because food challenges were not available in the MAS cohort. According to the estimated prevalence of FAs in the German population, a small proportion of controls may actually be affected by FAs. This would reduce the power in the case-control design applied for the association analysis of FLG mutations with different allergic foods. GOFA followed children at their routine clinical visits, which typically occur once per year. The end point of follow-up was therefore variable. In addition, some patients were lost to follow-up. Using the age at last positive challenge may therefore underestimate the duration of FA persistence because subsequent data were not available. On the other hand, loss to follow-up was more likely to occur in children with spontaneous tolerance development, which would lead to an underestimation of FA resolution. To alleviate this potential bias, we recontacted 96 children with HE allergy and 61 children with CM allergy who did not have a follow-up visit. Of 83 children who were HE-allergic and 57 children who were CM-allergic and who provided the requested information, 63% and 77%, respectively, reported spontaneous tolerance development, confirming that early loss to follow-up is more common among children who became tolerant.

We furthermore assessed how well the mutations under study represented the whole spectrum of LOF mutation in FLG. A total of 280 LOF mutations were reported in 129,196 individuals of European, non-Finnish ancestry in the gnomAD database. Most of them are personal mutations identified in a single individual. The 4 most common mutations analyzed in our study represent 82.0% of all haplotypes with an LOF mutation in Europeans (see Table E4 in this article’s Online Repository at www.jacionline.org). The fifth and sixth most common LOF mutations have a minor allele frequency of 0.05% and 0.04%, respectively. They are 5.5-fold less frequent compared with p.Ser3247Ter for which only 5 alleles were identified among our cases. Genotyping those is unlikely to significantly change our results. Hence, analyzing the 4 most common FLG LOF mutations is most efficient for association studies in populations of European ancestry.

Due to the ethnic homogeneity of the German study population, we were not able to investigate the role of FLG mutations in FA in other ethnic groups. Interestingly, the cumulative frequency of FLG LOF mutations is similar in Europeans (5.5% in non-Finnish Europeans), Africans (5.0% in African Americans), and Asians (5.5%, average between South and East Asians) (see Table E4 in this article’s Online Repository at www.jacionline.org). In addition, a comparable effect on eczema susceptibility was reported for Asian-specific and African American–specific LOF mutations in FLG. Moreover, equivalent biological effects were reported for LOF mutations identified in different populations, suggesting that they may also increase FA risk in other ethnic groups. Indeed, a Japanese study confirmed the association of 6 population-specific FLG LOF mutations with FA in Asians. A Turkish study investigating the 4 most common European mutations reported a combined allele frequency of 0.54% in patients with FAs, an over 20-fold lower frequency than in our study, which may point to different, population-specific FLG mutations in the Turkish. Confirmation studies particularly in non-European populations would be of great value.

Using the gold standard DBPCFC for FA diagnosis, we show an association of FLG LOF mutations with FA independent of the allergenic food tested and not confounded by eczema. Moreover, we demonstrate an FLG effect on the persistence of HE and CM allergy. This study does not only highlight the role of the impaired barrier in the development of any FA, it also demonstrates its impact on the long-term disease course. Hence, FLG LOF mutations should be considered when planning oral food rechallenges.
Clinical implications: In infants with HE or CM allergy, genotyping of \textit{FLG} LOF mutations identifies children at high risk of a persistent disease course.

REFERENCES

1. Tang ML, Mullins RJ. Food allergy: is prevalence increasing? Intern Med J 2017; 47:256-61.
2. Nwaru BI, Hickstein L, Panesar SS, Roberts G, Muraro A, Sheikh A, et al. Prevalence of common food allergies in Europe: a systematic review and meta-analysis. Allergy 2014;69:992-1007.
3. Muraro A, Werfel T, Hoffmann-Sommergruber K, Roberts G, Beyer K, Bindseil-Jensen C, et al. EAACI food allergy and anaphylaxis guidelines: diagnosis and management of food allergy. Allergy 2014;69:1008-25.
4. Grabbenhensich LB, Dolle S, Moneret-Vautrin A, Kohli A, Lange L, Spindler T, et al. Anaphylaxis in children and adolescents: the European Anaphylaxis Registry. J Allergy Clin Immunol 2016;137:1128-37.e1.
5. Braganzan SC, Acworth JP, McKinnon DR, Peake JF, Brown PA. Paediatric emergency department anaphylaxis: different patterns from adults. Arch Dis Child 2006;91:159-63.
6. Panesar SS, Javad S, de Silva D, Nwaru BI, Hickstein L, Muraro A, et al. The epidemiology of anaphylaxis in Europe: a systematic review. Allergy 2013;68:1353-61.
7. Sicherer SH, Wood RA, Vickery BP, Jones SM, Liu AH, Fleisher DM, et al. The natural history of egg allergy in an observational cohort. J Allergy Clin Immunol 2014;133:492-9.
8. Wood RA, Sicherer SH, Vickery BP, Jones SM, Liu AH, Fleisher DM, et al. The natural history of milk allergy in an observational cohort. J Allergy Clin Immunol 2013;131:805.e12.
9. Brown SJ, McLean WH. One remarkable molecule: filaggrin. J Invest Dermatol 2012;132:751-62.
10. Rawlings AV, Harding CR. Moisturization and skin barrier function. Dermatol Ther 2004;17(suppl 1):43-8.
11. Kim Y, Lim KM. Skin barrier dysfunction and filaggrin. Arch Pharm Res 2021;44:36-48.
12. Palmer CN, Irvine AD, Terron-Kwiatkowski A, Zhao Y, Liao H, Lee SP, et al. Common loss-of-function variants of the epidermal barrier protein filaggrin are a major predisposing factor for atopic dermatitis. Nat Genet 2006;38:441-6.
13. Marenholz I, Nickel R, Ruschendorf F, Schulz F, Esparza-Gordillo J, Kerscher T, et al. Filaggrin loss-of-function mutations predispose to phenotypes involved in the atopic march. J Allergy Clin Immunol 2006;118:866-71.
14. van den Oord RA, Sheikh A. Filaggrin gene defects and risk of developing allergic sensitisation and allergic disorders: systematic review and meta-analysis. BMJ 2009;339:b2433.
15. Smith SA, Dale BA. Immunologic localization of filaggrin in human oral epithelia and correlation with keratinization. J Invest Dermatol 1986;86:168-72.
16. Simon D, Radonic-Hosli S, Straussmann A, Yousefi S, Teuber SS, Burks AW, et al. Standardizing double-blind, placebo-controlled oral food challenges: American Academy of Allergy, Asthma & Immunology–European Academy of Allergy and Clinical Immunology PRACTALL consensus report. J Allergy Clin Immunol 2012;130:1260-74.
17. Hanifin JM, Rajka G. Diagnostic features of atopic dermatitis. Acta Derm Venereol 1980;60:44-7.
18. Williams HC, Burney PG, Strachan D, Hay RJ. The UK Working Party's diagnostic criteria for atopic dermatitis. II. Observer variation of clinical diagnosis and signs of atopic dermatitis. Br J Dermatol 1994;131:397-405.
19. Marenholz I, Suls M, Salamanca P, Schreiber R, Blümchen K, Schlags R, et al. Genome-wide association study identifies the \textit{SERPINE1} gene cluster as a susceptibility locus for food allergy. Nat Commun 2017;8:1056.
20. Bergmann RL, Bergmann KE, Lau-Schadenseder S, Luck W, Dannemann A, Bauer CP, et al. Atopic diseases in infancy. The German Multicenter Atopy Study (MAS-90). Pediatr Allergy Immunol 1994;5:19-25.
21. Lau S, Illi S, Sommerfeld C, Niggemann B, Bergmann RL, von Mutius E, et al. Early exposure to house-dust mite and cat allergens and development of childhood asthma: a cohort study. Multicentre Allergy Study Group. Lancet 2000;356:1392-7.
22. Purcell S, Neale B, Todd-Brown K, Hirsch J, Putschar D, et al. PLINK: a tool set for whole-genome association and population-based linkage analyses. Am J Hum Genet 2007;81:559-75.
23. Rich JT, Neely JG, Pinnel RC, Voelker CC, Nussenbaum B, Wang EW. A practical guide to understanding Kaplan-Meier curves. Otolaryng Head Neck Surg 2010;143:331-6.
24. Savage J, Sicherer S, Wood R. The natural history of food allergy. J Allergy Clin Immunol Pract 2016;4:196-203, quiz 4.
25. Sánchez-Borges M, Anstotegui I, Cox L. World Allergy Organization grading system for systemic allergic reactions: it is time to speak the same language when it comes to allergic reactions. Curr Treat Options Allergy 2019;6:388-95.
26. Berlydysh E, Goleva E, Bronoff AS, Hoffman BC, Ramirez-Gama MA, et al. Unique skin abnormality in patients with peanut allergy but no atopic dermatitis. J Allergy Clin Immunol 2021;147:361-7.e1.
27. Pepper AN, Sariahoom P, Casale TB. Emerging developments in the forefront of peanut oral immunotherapy. Curr Opin Allergy Clin Immunol 2021;21:263-8.
28. Dang TD, Peters RL, Koplin JJ, Dharmage SC, Gurrin LC, Ponsonby AL, et al. Egg allergy specific IgE diversity predicts resolution of egg allergy in the population cohort HealthNuts. Allergy 2019;74:318-26.
29. Caubet JC, Lin J, Ahrens B, Gimenez G, Bardina L, Niggemann B, et al. Natural filaggrin variants are associated with persistent atopic dermatitis in African Americans. J Investig Allergol Immunol 2018;138:1501-6.
46. Zhang H, Guo Y, Wang W, Shi M, Chen X, Yao Z. Mutations in the filaggrin gene in Han Chinese patients with atopic dermatitis. Allergy 2011;66:420-7.

47. Nomura T, Sandilands A, Akiyama M, Liao H, Evans AT, Sakai K, et al. Unique mutations in the filaggrin gene in Japanese patients with ichthyosis vulgaris and atopic dermatitis. J Allergy Clin Immunol 2007;119:434-40.

48. Hirota T, Nakayama T, Sato S, Yanagida N, Matsui T, Sugiura S, et al. Association study of childhood food allergy with genome-wide association studies-discovered loci of atopic dermatitis and eosinophilic esophagitis. J Allergy Clin Immuinol 2017;140:1713-6.

49. Acar NV, Cavkaytar O, Yilmaz EA, Buyuktiryaki B, Soyer O, Sahiner UM, et al. Rare occurrence of common filaggrin mutations in Turkish children with food allergy and atopic dermatitis. Turk J Med Sci 2020;50:1865-71.