Association of Filaggrin Variants with Asthma and Rhinitis: Is Eczema or Allergic Sensitization Status an Effect Modifier?

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Key Words

Filaggrin variants · Asthma · Rhinitis · Allergic sensitization · Eczema

Abstract

Background: Associations of variants of the filaggrin gene, i.e. *FLG* with asthma and rhinitis have been shown to be modulated by eczema status. However, it is unknown whether allergic sensitization status modifies this association. The aim of this study was to determine whether *FLG* variants need eczema and/or allergic sensitization as a necessary component to execute adverse effects on coexisting and subsequent asthma and rhinitis. **Methods:** In the Isle of Wight birth cohort, repeated measurements of asthma, rhinitis, eczema and allergic sensitization (documented by skin-prick tests) were taken in 1,456 children at the ages of 1, 2, 4, 10 and 18 years. Filaggrin haploinsufficiency was defined as having at least the minor allele of R501X, 2282del4 or S3247X variants. log-binomial regression models were used to test associations and statistical interactions. **Results:** *FLG* variants increased the risk of asthma [risk ratio (RR) 1.39, 95%

confidence interval (CI) 1.06–1.80] and rhinitis (RR 1.37, 95% CI 1.16–1.63). In the delayed-effects models, ‘*FLG* variants plus allergic sensitization’ and ‘*FLG* variants plus eczema’ increased the risk of subsequent asthma by 4.93-fold (95% CI 3.61–6.71) and 3.33-fold (95% CI 2.45–4.51), respectively, during the first 18 years of life. In contrast, neither eczema nor allergic sensitization in combination with *FLG* variants increased the risk of later rhinitis. **Conclusions:** Allergic sensitization and eczema modulated the association between *FLG* variants and asthma but not rhinitis. Our results imply that the mechanisms and pathways through which *FLG* variants predispose to an increased risk of asthma and rhinitis may be different.

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Introduction

The burden associated with allergic disorders including eczema, asthma and rhinitis is substantial and of public health importance. Early investigations indicated that a complex interplay between genetic, immunological and

environmental factors contributes to the development and maintenance of such allergic manifestations. Until recently, etiological research focused on the role of immune dysregulation in the pathogenesis of allergic disorders; however, a shift in the research paradigm towards understanding the contribution of a defective epidermal barrier rapidly emerged after the discovery of loss-of-function (LOF) variants in the filaggrin gene, *FLG* [1–3]. Filaggrin haploinsufficiency, defined as partial or complete loss of filaggrin (filament-aggregating protein) protein, is associated with the development of an impaired epidermal barrier that is characterized by increased allergen penetration and water permeability [4].

Thus far, *FLG* variants are the most replicated and strongest genetic risk factor for eczema [5]. They are also considered to be associated with asthma and rhinitis [6]. Of importance is that the association between *FLG* variants and asthma is stronger in the presence of eczema [5, 6]. However, this possible effect modification by eczema has not been demonstrated for the association between *FLG* variants and rhinitis [6]. Mechanisms that underlie the association of *FLG* variants with asthma and rhinitis are not well understood since the *FLG* is not expressed in the upper or lower airway epithelium [7, 8]. A proposed pathway is that cutaneous sensitization, facilitated by *FLG* variants, may lead to local and systematic inflammation at distant organs (i.e. the lung and nasal tissues) [2, 3].

While the concept of eczema leading to asthma in children who have loss-of-function variants in the *FLG* gene is attractive, research in this area has neglected the role of allergic sensitization, defined as the propensity to produce immunoglobulin E antibody responses to environmental and food antigens [9]. Only 1 study, on the German Multicenter Allergy Study birth cohort, has addressed allergic sensitization and reported an interaction between *FLG* variants and food sensitization in the pathogenesis of asthma among children with eczema [10]. Hence, further investigations on whether the associations of *FLG* variants with asthma and rhinitis are modified by eczema and/or allergic sensitization are needed so that preventive efforts can be directed to either clinical management of eczema or allergic sensitization or both.

We hypothesized that allergic sensitization, rather than eczema status, modifies the association of *FLG* variants with asthma and rhinitis. We tested statistical interactions to determine whether *FLG* variants plus allergic sensitization and/or *FLG* variants plus eczema jointly increased the risk of asthma and/or rhinitis. To this end,

analyzing longitudinal data from the Isle of Wight birth cohort, which covered childhood and adolescence prospectively, enabled us to determine whether *FLG* variants need allergic sensitization or eczema as a necessary component to be able to execute an adverse effect on coexisting and subsequent asthma and rhinitis.

Methods

Study Design and Participants

An unselected whole-population birth cohort (n = 1,536) was recruited in 1989 on the Isle of Wight, UK, to prospectively study the natural history of allergic conditions. After the exclusion of adoptions, perinatal deaths and refusal of follow-up, 1,456 (95%) children were enrolled, with follow-up assessments conducted at 1, 2, 4, 10 and 18 years of age. Ethics approvals were obtained from the Isle of Wight Local Research Ethics Committee (NRES Committee South Central – Southampton B) at recruitment and for the subsequent follow-ups (06/Q1701/34). At each follow-up, validated questionnaires including the International Study of Asthma and Allergy in Childhood (ISAAC) questionnaire [11] were completed on allergic disorders, demographic attributes and exposure to environmental factors.

Phenotypes

In all assessments of the Isle of Wight birth cohort, eczema was defined as chronic or chronically relapsing itchy dermatitis lasting more than 6 weeks with characteristic morphology and distribution [12], according to the criteria of Hanifin and Rajka [13]. For asthma, at the 1-, 2- and 4-year follow-ups, the medical investigator determined the presence of asthma based on wheeze frequency over the last 12 months and treatment given for asthma or asthma-related symptoms. At the 10- and 18-year follow-ups, asthma was defined as having ‘ever had asthma’ and either ‘wheezing or whistling in the chest in the last 12 months’ or ‘current treatment for asthma’, using the ISAAC questionnaire [11]. Rhinitis was defined by a positive response to: ‘In the past 12 months have you had a problem with sneezing, or a runny or a blocked nose when you did not have a cold or the flu?’ [14].

To determine allergic sensitization status, skin-prick tests (SPT) at ages 1 and 2 years were performed on children with any symptoms of eczema, asthma or rhinitis. We combined SPT results for ages 1 and 2 years since this was a relatively short time period; these are henceforth referred to as SPT at 1 or 2 years. At 4, 10 and 18 years, regardless of symptoms, SPT to a standard battery of common allergens were performed on most children attending the research center (ALK-Albello, Horsholm, Denmark). The inhalant allergens tested were the house-dust mite, cat, dog, *Alternaria alternata*, *Cladosporium herbarium*, grass pollen mix and tree pollen mix. The food allergens tested were cow’s milk, soya, hen’s egg, peanut and cod. Positive and negative controls were included. Allergic sensitization was defined by having a positive SPT to at least one allergen test with a mean wheal diameter of 3 mm greater than the negative control. Since allergic sensitization is a dynamic rather than a completely stable phenotype, we used the concurrent and/or preceding status and thus allowed the risk to change over time.

FLG Genotyping

Blood and/or saliva samples, from which genomic DNA was isolated, were collected from children at the age of 10 and/or 18 years. The DNA samples were interrogated using GoldenGate Genotyping Assays (Illumina Inc., San Diego, Calif., USA) on the BeadXpressVeracode platform (Illumina Inc.) per Illumina's protocol. Individuals carrying the minor allele for at least one of the *FLG* variants R501X, 2282del4, or S3247X were classified as having filaggrin haploinsufficiency. The R2447X variant was genotyped too, but none of the study participants carried the minor allele. Detailed information on genotyping is provided by Ziyab et al. [15].

Statistical Analysis

Since the proposed effect modifiers (i.e. allergic sensitization and eczema) and the two outcomes (i.e. asthma and rhinitis) were measured repeatedly at ages 1 or 2 years, 4, 10 and 18 years, we applied the generalized estimating equation (GEE) method with first-order autoregressive covariance matrix to account for the correlated observations and the within-child effect of the repeated measurements [16]. Risk ratios (RR) and their 95% confidence intervals (95% CIs) were estimated by applying log-binomial regression models using the GENMOD procedure in SAS 9.3 (SAS, Gary, N.C., USA). To determine whether the RR of the association of *FLG* variants with asthma and rhinitis differ according to the presence or absence of the proposed effect modifiers, we evaluated the association in the total study sample and subsamples based on the status of the possible effect modifiers.

Interaction terms, on a multiplicative scale, were used to test the additional effect of two co-occurring risk factors on the health outcomes above and beyond their individual effects. Also, to evaluate whether the effect of *FLG* variants across levels of the effect modifiers were statistically significantly different, we included interaction terms in separate regression models (model 1: *FLG* variants $\times$ eczema and model 2: *FLG* variants $\times$ allergic sensitization). To decipher which interactive effect was more pronounced, a regression model including both interaction terms (model 3: *FLG* variants $\times$ eczema and *FLG* variants $\times$ allergic sensitization) was evaluated. Henceforth, we refer to models 1, 2 and 3 as the 'concurrent models' since the effect modifiers and the outcomes coexisted at the same time. In the case of a possibly statistically significant interaction term ($P_{\text{interaction}} < 0.1$), the 'combined effect', referring to the joint impact of two individual risk factors plus their interaction on the occurrence of the outcome, was estimated. We repeated the previous analyses while stratifying by sex to determine if sex-specific effects existed. In all GEE models, sex and age at follow-up were included as potential confounders.

In addition to the concurrent-effects models, we tested the interactions in 'delayed effect' models to investigate whether the time order of risk and response supported the concurrent-effects model findings. The delayed-effects analysis helps to determine whether the interaction effects contribute to the developmental process of asthma and rhinitis. To this end, we analyzed whether *FLG* variants interact with preceding allergic sensitization or eczema on the risk of subsequent asthma and rhinitis. For example, the interaction of *FLG* variants with allergic sensitization status at the age of 1 or 2 years on the risk of asthma at the age of 4 years (*FLG* variants $\times$ SPT results at 1 or 2 years $\rightarrow$ asthma at 4 years) was evaluated. The prevalence of the clinical phenotype (e.g. asthma at 4 years), which was used in the delayed-effects models, included new occur-

Table 1. Characteristics of the study population

Attributes	% (n/total)
Sex	
Male	51.2 (786/1,536)
Eczema at	
1 or 2 years	14.2 (196/1,377)
4 years	11.9 (145/1,214)
10 years	13.7 (186/1,359)
18 years	12.3 (161/1,307)
Asthma at	
1 or 2 years	14.3 (197/1,377)
4 years	14.9 (181/1,214)
10 years	14.7 (201/1,368)
18 years	17.7 (231/1,305)
Rhinitis at	
1 or 2 years	15.8 (217/1,377)
4 years	5.6 (65/1,214)
10 years	22.6 (308/1,362)
18 years	35.8 (468/1,309)
<i>FLG</i> variants ^a	
R501X	4.1 (47/1,161)
2282del4	4.6 (54/1,168)
S3247X	1.6 (18/1,165)
Combined	10.3 (118/1,150)
Allergic sensitization ^b at	
1 or 2 years	20.6 (106/515)
4 years	19.6 (192/982)
10 years	26.9 (279/1,036)
18 years	41.4 (353/853)

^a Analyses were conducted using the combined carrier frequency of 10.3%.

^b Defined by having one or more positive SPT results at the respective assessment.

rences and persistence of the disease. Therefore, we constructed 3 delayed periods (1 or 2 to 4 years, 4–10 years and 10–18 years) to determine if the presence of both *FLG* variants and preceding allergic sensitization or preceding eczema influences the positive transition, defined as the change in disease status from disease-free to diseased and the persistence of the disease in 2 consecutive follow-ups [17].

Results

Description of Study Population

Of the 1,456 children enrolled in the study, 1,377 were followed at the age of 1 or 2 years, 1,214 at 4 years, 1,368 at 10 years and 1,309 at 18 years. The period prevalence ranged between 11.9–14.2% for eczema, 14.3–17.7% for asthma and 5.6–35.8% for rhinitis (table 1). At the age of

Fig. 1. Concurrent association of *FLG* variants with asthma in the total study sample and subsamples: longitudinal analysis covering 1 or 2 years to 18 years of age. The prevalence of asthma among those with *FLG* LOF variants and those with wild-type genotype (*FLG* WT) is shown. k = Number of repeated measurements.

1 or 2 years, SPT were performed on symptomatic children (n = 515), 20.6% of whom had at least one positive SPT response. At all other ages, the majority of participants underwent SPT, with a positive result in 19.4% at 4 years, 26.9% at 10 years and 41.4% at 18 years of age. Genotype frequencies of *FLG* variants (table 1) were concordant with the Hardy-Weinberg equilibrium.

FLG Variants and Asthma

In the total study sample, *FLG* variants increased the risk of repeated occurrence of asthma from 1 or 2 years to 18 years of age (RR 1.39, 95% CI 1.06 – 1.80; fig. 1). In the presence of eczema, the effect size increased (RR 1.56, 95% CI 1.10–2.21); in the absence of eczema, the magnitude of the association between *FLG* variants and asthma was reduced and lost statistical significance (RR 1.26, 95% CI 0.93–1.69). This association was noticeably modified when stratifying based on the presence (RR 1.49, 95% CI 1.14–1.94) or absence (RR 0.76, 95% CI 0.48–1.21) of allergic sensitization.

To determine whether the observed differences in the associations were statistically significant, statistical interactions on a multiplicative scale were evaluated in concurrent-effects models. There was no evidence for an interaction between *FLG* variants and eczema on the risk for asthma (interaction effect RR 1.18, 95% CI 0.78–1.79, $p_{\text{interaction}} = 0.429$; fig. 1). However, a statistically significant interaction was found between *FLG* variants and allergic sensitization on the risk for asthma (interaction effect RR 2.00, 95% CI 1.22–3.28, $p_{\text{interaction}} = 0.006$; fig. 1). The combined effect of *FLG* variants and allergic sensi-

zation increased the risk of asthma by 3.63-fold (95% CI 2.81–4.70). Furthermore, to determine which interactive effect is more important, we simultaneously included both interaction terms in 1 regression model. Results showed that the interaction between *FLG* variants and allergic sensitization remained statistically significant after adjusting for the interactive effect between *FLG* variants and eczema (data not shown). However, the latter did not gain statistical significance.

We retested the results of the aforementioned concurrent-effects models, using delayed-effects models. In these models, we used the preceding cumulative SPT results (or eczema status), i.e. all SPT results (or eczema status) from assessments that were prior to the outcome assessment, when testing the interaction between *FLG* variants and allergic sensitization (or eczema) on the risk of subsequent asthma. Repeated-measurements analysis demonstrated a statistically significant interaction between *FLG* variants and preceding allergic sensitization on the risk of subsequent asthma (RR_{interaction} 1.58, $p_{\text{interaction}} = 0.013$). The results of the delayed-effects models show that the combined effect of preceding allergic sensitization with *FLG* variants increased the risk of asthma in the next exam at each delay period (fig. 2a). For instance, the presence of both *FLG* variants plus a positive SPT at 1 or 2 years and/or 4 years increased the risk of asthma at 10 years (RR 7.22, 95% CI 4.84–10.80). Although the results of the concurrent-effects model (fig. 1) did not show an interaction between *FLG* variants and eczema, the delayed-effects models demonstrated interactive effects between *FLG* variants and preceding eczema

Fig. 2. a Delayed-effects models exploring the individual and combined effects of *FLG* variants and preceding allergic sensitization status (documented by SPT) on asthma development. The independent effects of the *FLG* variants, negative SPT and *FLG* LOF, positive SPT and *FLG* wild-type and their combined effect, positive SPT and *FLG* LOF in the development of asthma are shown for different ages. We modeled the effect of preceding SPT results on later asthma. For instance, having at least one positive SPT between the ages of 1 or 2 years and 10 years was used as a predictor of asthma at 18 years. **b** Delayed-effects models exploring the individual and combined effects of *FLG* variants and preceding eczema status on asthma development. The independent effects of the *FLG* variants, negative for eczema and *FLG* LOF, positive for eczema *FLG* wild type and their combined effect, positive for eczema and *FLG* LOF in the development of asthma are shown for different ages. We modeled the effect of preceding eczema results

on later asthma. For instance, having at least one positive eczema diagnosis between the ages of 1 or 2 years and 10 years was used as a predictor of asthma at 18 years. Eczema – = Negative for eczema; Eczema + = positive for eczema; *FLG* WT = *FLG* wild-type genotype; k = number of repeated measurements; n = number of children; SPT – = negative SPT; SPT + = positive SPT. * $p_{\text{interaction}} < 0.1$; † $p_{\text{interaction}} < 0.05$, indicating that the interaction effect, i.e. SPT × *FLG* (a) and Eczema × *FLG* (b) is possibly statistically significantly different from the null value of 1, suggesting interaction on a multiplicative scale. ‡ $p < 0.05$, indicating that the RR of the given column is statistically significantly different from the column with neither of the risk factors, i.e. SPT – and *FLG* WT (a) and Eczema – and *FLG* WT (b). The presence of both an asterisk (*) or double dagger (‡) and dagger (†) signifies that the combined effect of the two risk factors is above and beyond their individual effects.

Table 2. Stratification of prevalence, positive transition and persistence of asthma based on *FLG* variants and preceding allergic sensitization and eczema status: comparing the number of patients with and without the 2 risk factors

	<i>FLG</i> WT and SPT –	<i>FLG</i> LOF and SPT +	p value ^a	<i>FLG</i> WT and eczema –	<i>FLG</i> LOF and eczema +	p value ^a
<i>Prevalence</i>						
At age 4 years	18.4 (57/310)	50.0 (9/18)	0.003	11.9 (86/725)	52.0 (5/6)	<0.001
At age 10 years	7.9 (47/595)	60.0 (18/30)	<0.001	11.2 (91/811)	51.5 (17/33)	<0.001
At age 18 years	10.3 (62/604)	54.8 (23/42)	<0.001	16.0 (118/739)	51.3 (20/39)	<0.001
<i>Positive transition^b</i>						
At 1 or 2 to 4 years	8.5 (19/223)	50.0 (6/12)	<0.001	8.5 (53/635)	42.1 (8/19)	<0.001
At 4–10 years	4.3 (23/532)	31.3 (5/16)	<0.001	7.0 (44/632)	29.4 (5/17)	0.006
At 10–18 years	6.7 (37/550)	23.8 (5/21)	0.014	9.3 (59/639)	14.3 (3/21)	0.437
<i>Persistence^c</i>						
At 1 or 2 to 4 years	43.7 (38/87)	50.0 (3/6)	0.542	36.7 (33/90)	83.3 (5/6)	0.031
At 4–10 years	38.7 (24/62)	92.9 (13/14)	<0.001	44.7 (38/85)	80.0 (12/15)	0.023
At 10–18 years	56.8 (25/44)	85.7 (18/21)	0.026	68.3 (56/82)	94.4 (17/18)	0.037

Figures denote the proportion of asthma as a percentage (number/total). Eczema – = Negative for eczema; eczema + = positive for eczema; *FLG* WT = *FLG* wild-type genotype; SPT – = negative SPT; SPT + = positive SPT.

^a Comparisons of proportions across the 2 exposure groups were derived by the Fisher exact test.

^b The change in disease status from asthma-free to asthma in 2 consecutive assessments.

^c The proportion of individuals who had asthma at 2 consecutive assessments.

on the risk of subsequent asthma (repeated measurements analysis: $RR_{\text{interaction}} 1.96$, $p_{\text{interaction}} = 0.006$; fig. 2b). The repeated measurement analysis shows that the combined effect of *FLG* variants and preceding eczema, on average, increased the risk of later asthma during the first 18 years of life ($RR 3.33$, 95% CI: 2.45–4.51).

To further understand whether the observed interactions between *FLG* variants and preceding allergic sensitization and eczema influence new occurrences and/or the persistence of asthma, we calculated the proportions of positive transitions (defined as the change in disease status from disease-free to diseased in 2 consecutive follow-ups) and persistence among those with and without both risk factors (table 2). The proportion of positive transition was more frequent among those with *FLG* variants and allergic sensitization than in those with neither risk factor across all transition periods. For instance, in the presence of both *FLG* variants and allergic sensitization, 5/16 (31.3%) of the children without asthma at the age of 4 years developed asthma at the age of 10 years, but only 23/532 (4.3%) of those with neither risk factor developed asthma at 10 years. Furthermore, the presence of both risk factors influenced the persistence of asthma (table 2). For example, 18/21 (85.7%) participants with both risk factors who had asthma at the age of 10 years continued to have asthma at 18 years of age, compared to 25/44

(56.8%) of those with neither risk factor. Similarly, the presence of both *FLG* variants and preceding eczema influenced the positive transition and persistence of asthma.

FLG Variants and Rhinitis

FLG variants were associated with an increased risk for rhinitis from age 1 or 2 years to 18 years in the total study sample of repeated measurements ($RR 1.37$, 95% CI 1.16–1.63; fig. 4). In both the presence ($RR 1.57$, 95% CI 1.23–2.00) and absence ($RR 1.28$, 95% CI 1.04–1.57) of eczema, the association between *FLG* variants and rhinitis was statistically significant. However, we observed a statistically significant association between *FLG* variants and rhinitis only in the presence of allergic sensitization ($RR 1.34$, 95% CI 1.17–1.53), and not in its absence ($RR 1.13$, 95% CI 0.82–1.55; fig. 4).

To test if the observed heterogeneous effects across the levels of the potential effect modifiers were statistically different, we tested multiplicative statistical interactions in the concurrent-effects models. There was no evidence for an interactive effect between *FLG* variants and eczema (interaction effect $RR 1.15$, 95% CI 0.83–1.60, $p_{\text{interaction}} = 0.409$; fig. 4) or between *FLG* variants and allergic sensitization (interaction effect $RR 1.07$, 95% CI 0.76–1.51, $p_{\text{interaction}} = 0.698$; fig. 4) on the risk for rhinitis. Concordant

Fig. 3. a Delayed-effects models exploring the individual and combined effects of *FLG* variants and preceding allergic sensitization status (documented by SPTs) on rhinitis development. The independent effects of *FLG* variants, negative SPT and *FLG* LOF, positive SPT and *FLG* WT and their combined effect, positive SPT and *FLG* LOF in the development of rhinitis are shown for different ages. We modeled the effect of preceding SPT results on later rhinitis. For instance, having at least one positive SPT between 1 or 2 years and 10 years was used as a predictor of rhinitis at 18 years. **b** Delayed-effects models exploring the individual and combined effects of *FLG* variants and preceding eczema status on rhinitis development. The independent effects of *FLG* variants, negative for eczema and *FLG* LOF, positive for eczema and *FLG* WT and their combined effect, positive for eczema and *FLG* LOF in the development of rhinitis are shown for different ages. We modeled the effect of preceding eczema results on later rhinitis. For instance, having at least one positive eczema diagnosis between 1 or 2 years and 10 years was used as a predictor of rhinitis at 18 years. Eczema – = negative for eczema; Eczema + = positive for eczema; *FLG* WT = *FLG* wild-type genotype; k = number of repeated measurements; n = number of children; SPT – = negative SPT; SPT + = positive SPT. † $p < 0.05$, indicating that the RR of the given column is statistically significantly different from the column with neither risk factor, i.e. SPT – and *FLG* WT(**a**) and Eczema- and *FLG* WT (**b**).

with the results of the concurrent-effects models, the delayed-effects models did not show any interactions between *FLG* variants and preceding allergic sensitization or eczema on the risk of subsequent rhinitis (fig. 3).

Sex Differences and Time Trends

Separate analyses for each sex were performed, with the results for boys and girls being similar (data not shown) and in agreement with the results of analyzing both sexes together in the repeated-measurement (GEE) analysis. Furthermore, at each follow-up, we analyzed

boys and girls separately to determine whether the effects differed across both time and sex. The obtained results indicated that neither age at the exam nor sex influenced our results.

Discussion

The objective of this study was to determine whether eczema and/or allergic sensitization status act as effect modifiers for the association of *FLG* variants with asthma

Fig. 4. Concurrent association of *FLG* variants with rhinitis in the total study sample and subsamples: longitudinal analysis covering 1 or 2 years to 18 years of age. The prevalence of rhinitis among those with *FLG* LOF variants and those with wild-type genotype (*FLG* WT) is shown. k = Number of repeated measurements.

and rhinitis. In this study, *FLG* variants increased the risk of asthma and rhinitis during the first 18 years of life in the total study sample. In the concurrent-effects models, interaction between *FLG* variants and allergic sensitization resulted in a combined effect that increased the risk of coexisting asthma by 3.63-fold. The delayed-effects models, which take the time order of risk factors and disease occurrences into account, supported the findings of the concurrent-effects models with regard to allergic sensitization and *FLG* variants. They also showed that *FLG* variants interact with preceding eczema status on the development of subsequent asthma, indicating that both allergic sensitization and eczema act as effect modifiers for the association between *FLG* variants and asthma. In contrast, neither eczema nor allergic sensitization modified the association of *FLG* variants with rhinitis in a statistically significant manner.

The association between *FLG* variants and asthma among those with eczema was first reported by Palmer et al. [1]. Subsequently, several studies have replicated this association and added a possible association between *FLG* variants and rhinitis [5, 6]. Our results further support the general agreement that *FLG* variants are associated with asthma in the presence of eczema. We further demonstrated that both preceding allergic sensitization and eczema modified the association between *FLG* variant and asthma through multiplicative interaction (fig. 2). These findings indicate that *FLG* variants need eczema or allergic sensitization to execute their adverse effects on asthma. The finding of an interaction between *FLG* variants and allergic sensitization in this study improves our

understanding of the possible link between the genetics of the epidermal barrier (i.e. *FLG* variants) and a respiratory disorder (i.e. asthma). Such an observation further supports the hypothesis that cutaneous-sensitization priming, facilitated by *FLG* variants, may migrate to the airways and cause local and systematic inflammation [18]. Although previous studies broadly suggested such a pathway, most of them did not take the status of allergic sensitization into account. An exception is the study by Marenholz et al. [10], showing an interaction between *FLG* variants and food sensitization on the risk of asthma in children with eczema; their analytical sample was restricted to children with eczema, however.

In the delayed-effects models, we used the prevalence of asthma as the outcome, which does not distinguish between new occurrences and persistence of asthma. In additional descriptive analysis, we demonstrated that the presence of both *FLG* variants and preceding allergic sensitization or preceding eczema influenced the new occurrences (positive transition) and persistence of asthma. Hence, our data suggest that the presence of both risk factors ('*FLG* variants and preceding allergic sensitization' or '*FLG* variants and preceding eczema') plays an important role in the development and persistence of asthma.

Attempts using the composite of the 'atopic asthma' phenotype were conducted to investigate whether allergic sensitization is the link between *FLG* variants and asthma. Inconsistent results for the association of *FLG* variants with 'atopic asthma' have been reported [19–22]. These could be attributed to the use of the composite 'atopic asthma' phenotype, which does not clearly define the ref-

erence (no-risk) group. With this approach, investigators tend to group 'non-atopic asthmatic' and 'atopic without asthma' participants together in the reference group. Therefore, when assessing the association of *FLG* variants with 'atopic asthma', one should be aware that the reference group could include atopic participants, which could result in distorted assessments. The advantage of our approach, using two separate risk factors, over the composite 'atopic asthma' phenotyping, is that we were able to estimate the additional effect due to interaction (*FLG* variants $\times$ allergic sensitization) that is above and beyond their independent effects, thereby allowing us to determine whether the combined effect of both risk factors is more than just the multiplication of their independent effects.

There is conflicting evidence in the literature as to whether the association of *FLG* variants with rhinitis is modulated by eczema status. The majority of previous studies found a stronger link between *FLG* variants and rhinitis in the presence of coexisting eczema when compared to their association in the absence of concurrent eczema [6, 20, 23–25]. Another investigation reported a link between *FLG* variants and the 'persistent allergic rhinitis' phenotype [21]. Controversially, however, a significant link between *FLG* variants and the composite 'allergic rhinitis' phenotype adjusted for eczema status has also been reported [19]. Our analyses did not reveal an interaction between eczema ($p_{\text{interaction}} = 0.409$) or allergic sensitization ($p_{\text{interaction}} = 0.698$) with *FLG* variants on the risk of rhinitis in the repeated-measurements analysis. Our results of an interactive effect between *FLG* variants and allergic sensitization on the risk of asthma and the lack of such interaction on the risk of rhinitis suggest that the pathways through which *FLG* variants predispose to asthma and rhinitis may be different. For instance, the expression of *FLG* was not detected in the human bronchial epithelium, but it has been found to be expressed in the nasal vestibule [7, 19].

The major strengths of our prospective, 18-year study are the repeated phenotyping, the objective assessments of allergic sensitization status and a low loss to follow-up (5–17%). Moreover, the majority (80%) of the study participants were genotyped for *FLG* variants common in populations of European ancestry [26]. We showed, previously, that the genotyped study participants did not differ from the total cohort with regard to multiple characteristics [15]. Hence, there is no indication of selection bias that could pose a threat to the validity of our study. Misclassification of eczema cases was minimal because a high proportion of the subjects showed typical manifesta-

tions of eczema in the usual locations, i.e. antecubital or popliteal fossae, ankles, face or neck at 1 year ($n = 97$), 2 years ($n = 91$), 4 years ($n = 75$), 10 years ($n = 86$) and 18 years (76%) [27]. To ensure reproducibility of results, the data were randomly divided into two equal parts and analyses were repeated (see suppl. table S1, www.karger.com/doi10.1159/000365990). This post hoc confirmatory step showed substantial consistency in the results with regard to interaction effect sizes across the two subsamples and the total analytical sample.

Our definition of asthma and rhinitis symptoms in early life is a possible limitation of the study. Our asthma conclusion at the age of 10 and 18 years followed the ISAAC criteria [11], which, at that time, was not available for assessments at the age of 1, 2 and 4 years. Although slightly different methods were used to define asthma, in a previous report we showed that a minor change in this definition over time did not influence the validity of our classification [17]. In addition, since it is difficult to differentiate between infectious and other forms of rhinitis in infancy, the elevated prevalence at 1 or 2 years might have been influenced by misclassifying 'viral induced infectious rhinitis' [14]. However, by applying repeated-measurements analysis including assessments later in childhood and adolescence, we believe that the influence of such a misclassification on the overall results of the study was minimal, since similar results were obtained when we reran our analyses and excluded the 1- or 2-year data. Moreover, since SPTs at age 1 or 2 years were performed on children with any symptom, when excluding the 1- or 2-year results, we could show that a selection resulting in an underestimation of RRs might have been induced. Although proportions of SPT positivity at ages 1 or 2 years (20.6%) and 4 years (19.6%; SPT performed irrespective of symptoms) were similar, RRs associating allergic sensitization at age 1 or 2 years with outcomes (i.e. eczema, asthma and rhinitis) at the same age tended to be smaller than RRs relating allergic sensitization at the age of 4 years with outcomes at the same age (data not shown), because the reference group also had some symptoms. Hence, the estimated RRs at age 1 or 2 years might be underestimated; however, the extent of the possible selection bias was minimal because our results did not change noticeably when we excluded the data of the follow-up at 1 or 2 years from the analyses.

The use of a slightly relaxed statistical significance threshold ($p_{\text{Interaction}} < 0.1$) for detecting interaction-effects is further supported by the fact that the majority of epidemiological studies lack sufficient power to detect higher-order terms [28]. However, applying repeated-

measurements analyses improved the statistical power for detecting interaction effects. For instance, in the delayed-effects models with separate analyses for the 3 delayed periods (1 or 2 years to 4 years, 4–10 years and 10–18 years), the *p* values for the interaction terms of *FLG* variants and eczema on the risk of subsequent asthma were between 0.05 and 0.1; however, the results of the repeated-measurements models combining the 3 delayed periods yielded interaction terms with *p* values that were highly statistically significant (e.g. *FLG* variants $\times$ preceding eczema: $p_{\text{interaction}} = 0.006$). Testing the interaction effects in 3 delayed-effect periods might have identified false-positive interaction effects due to a relaxed *p* value of 0.1, but the statistical significance observed in the repeated-measurements analysis reduced the possibility of finding false-positive results.

In conclusion, our results indicate that allergic sensitization and eczema status are effect modifiers of the association between *FLG* variants and asthma. The combined effects of ‘preceding allergic sensitization and *FLG* variants’ and ‘preceding eczema and *FLG* variants’ increased the risk of subsequent asthma by 4.93-fold and 3.33-fold, respectively, in the first 18 years of life. These findings

suggest that *FLG* variants need allergic sensitization or eczema to execute adverse effects on asthma. In contrast, neither eczema nor allergic sensitization status statistically significantly modulated the association of *FLG* variants with rhinitis. Our results suggest that the mechanisms and pathways through which *FLG* variants, (representing an impaired epidermal barrier) predispose to an increased risk of asthma and rhinitis may be different. Future studies confirming our reported observations and exploring the differential etiological pathways underlying the development of asthma and rhinitis are needed to better identify and stratify patients who share similar risk characteristics.

Acknowledgements

We thank the participants and their families who have helped us with this project over the last two decades. We would like to acknowledge the help of all the staff at The David Hide Asthma and Allergy Research Centre in undertaking the 18-year and previous assessments of the 1989 Isle of Wight birth cohort. The 18-year assessment and other analyses were funded by grants from the National Institute of Health, USA (R01 HL082925 and R01AI091905).

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