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Allergic sensitization and filaggrin variants predispose to the comorbidity of eczema, asthma, and rhinitis: results from the Isle of Wight birth cohort

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Abstract

Background—Allergic sensitization and filaggrin gene (*FLG*) variants are important risk factors for allergic disorders; however, knowledge on their individual and interactive effects on the coexistence of eczema, asthma, and rhinitis is lacking.

Objective—This study aimed at investigating the single and combined effects of allergic sensitization and *FLG* variants on the development of single and multiple allergic disorders.

Methods—The Isle of Wight Birth Cohort (n = 1,456) has been examined at 1, 2, 4, 10, and 18 years of age. Repeated measurements of eczema, asthma, rhinitis, and skin prick tests were available for all follow-ups. *FLG* variants were genotyped in 1,150 participants. Associations of allergic sensitization and *FLG* variants with single and multiple allergic disorders were tested in log-binomial regression analysis.

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Conflict of interest

The authors declare no conflicts of interest that could raise questions about a paper's credibility.

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Results—The prevalence of eczema-, asthma-, and rhinitis-only ranged from 5.6% to 8.5%, 4.9% to 10.2%, and 2.5% to 20.4%, respectively, during the first 18 years of life. The coexistence of allergic disorders is common, with approximately 2% of the population reporting the comorbidity of “eczema, asthma, and rhinitis” during the study period. In repeated measurement analyses, allergic sensitization and *FLG* variants, when analyzed separately, were associated with having single and multiple allergic disorders. Of particular significance, their combined effect increased the risk of “eczema and asthma” (RR = 13.67, 95% CI: 7.35 – 25.42), “asthma and rhinitis” (RR = 7.46, 95% CI: 5.07 – 10.98), and “eczema, asthma, and rhinitis” (RR = 23.44, 95% CI: 12.27 – 44.78).

Conclusions and Clinical Relevance—The coexistence of allergic disorders is frequent and allergic sensitization and *FLG* variants jointly increased risk of allergic comorbidities, which may represent more severe and complex clinical phenotypes. The interactive effect and the elevated proportion of allergic comorbidities associated with allergic sensitization and *FLG* variants emphasize their joint importance in the pathogenesis of allergic disorders.

Introduction

Allergic disorders, including eczema, asthma, and rhinitis, pose social and economic burden on individuals, families, and societies [1, 2]. Worldwide, the lifetime prevalence of eczema symptoms (e.g., itchy rash) is estimated to be 15% to 30% among children and 2% to 10% among adults [3]. Similarly, the prevalence of asthma (up to 25%) and rhinitis (up to 30%) is high [4–6]. Natural history investigations have demonstrated close and complex relationships between these allergic disorders [7–12]. However, mechanisms and pathways underlying their development is an area of ongoing scientific dispute.

The “allergic march” concept suggests that allergic disorders develop in a sequential pattern, starting with eczema in early infancy and later in childhood developing asthma and rhinitis [13]. However, supporting replicable evidence and consensus is far from complete [14–16]. An opposing concept is the “coexistence” of allergic morbidities [14, 17, 18]. A report based on the German Multicenter Allergy Study showed that single occurrence of eczema early in life does not result in an allergic march, but the coexistence of eczema and wheezing predicted asthma [18]. Results from the BAMSE birth cohort showed that the comorbidity of allergic disorders is frequent during the first 12 years of life [17]. Such observations speak in favor of coexistence of allergic morbidities instead of a progressive development. The interrelationship between these conditions and the risk factors that predispose individuals to develop multiple allergic disorders is an open field for research.

Interplay between genetic, environmental, and immunological factors is considered to contribute to the pathogenesis of allergic disorders [19]. Allergic sensitization, defined as the susceptibility to produce immunoglobulin E (IgE) antibodies in response to antigens, is widely-considered as a common thread linking various manifestations of allergic disorders [20, 21]. Likewise, loss-of-function variants in the filaggrin gene (*FLG*) resulting in an impaired epidermal barrier have rapidly emerged as common risk factor for the development of allergic disorders [22]. However, there is a gap in understanding of the respective contribution of *FLG* variants and allergic sensitization on the development of allergic

comorbidities. Moreover, the joint role of *FLG* variants and allergic sensitization on the coexistence of eczema, asthma, and rhinitis has not been previously investigated. Using data from the Isle of Wight (IOW) birth cohort, we aimed at determining the single and combined effects of *FLG* variants and allergic sensitization on the development of single and multiple (coexisting) allergic disorders.

Materials and 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 exclusion of adoptions, perinatal deaths, and refusal for follow-up, written informed consent was obtained from parents to enroll 1,456 (95%) newborns, 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 (now named the National Research Ethics Service, NRES Committee South Central – Southampton B) at recruitment and for the subsequent follow-ups (06/Q1701/34).

Phenotypes

In all assessments of the IOW birth cohort, eczema was defined as chronic or chronically relapsing, itchy dermatitis lasting more than 6 weeks with characteristic morphology and distribution [23], following Hanifin and Rajka criteria [24]. For asthma, at the 1, 2, and 4-year follow-ups, the medical investigator determined the presence of asthma based on wheeze 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”, following the International Study of Asthma and Allergies in Childhood (ISAAC) criteria [25]. Rhinitis was defined by a problem with sneezing, or a runny or a blocked nose without a cold or the flu in the last 12 months [8]. Since the 1-year and 2-year follow-up data were collected in a relatively small time window, we combined them for analytic purposes (reported as 1-or-2 years).

To determine allergic sensitization, skin prick tests (SPTs) at ages 1 and 2 years were performed on children with any symptoms of eczema, asthma, or rhinitis. Parallel to the disorders, we combined the SPTs at ages 1 and 2 years (reported as 1-or-2 years). At 4, 10, and 18 years, regardless of symptoms, SPTs were performed on most children attending the research center using 14 common food and aeroallergens (ALK-Albello, Horsholm, Denmark) [26]. Allergic sensitization was defined by having a positive SPT to at least one test with mean wheal diameter of 3 mm greater than the negative control. Since allergic sensitization is a dynamic phenotype, we used the concurrent status and thus allowed the risk to change over time.

FLG genotyping

Blood and/or saliva samples were collected at ages 10 and/or 18 years from which genomic DNA was isolated. DNA samples were interrogated using GoldenGate Genotyping Assays

on the BeadXpressVeracode platform (Illumina, Inc, SanDiego, CA) 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. Detailed information on genotyping is provided by Ziyab *et al* [27].

Statistical analysis

The 12-month period prevalence of eczema, asthma, rhinitis, and their comorbidities combinations was determined for each follow-up. Combinations of three disorders resulted in eight non-overlapping comorbidities groups (Table 1). To estimate the single and combined effects of the risk factors, *FLG* variants and repeated measures of allergic sensitization, on allergic disorders, generalized estimating equations (GEE) were used when estimating the covariance matrix to account for correlated observations and the within-child effect of the repeated measurements [28]. 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, NC, USA). The "no allergic disorder" category formed the reference category. The main effects of *FLG* variants and allergic sensitization on the risk for single allergic disorders and comorbidities were estimated separately (single risk factor models). In a full model, the two main plus their interaction effect (*FLG* variants $\times$ allergic sensitization) were estimated. We repeated the aforementioned analysis while excluding the 1-or-2 years follow-up data from the GEE analysis to estimate whether possible misclassifications of phenotypes at early life biased our results. In the case of a possibly statistically significant interaction ($P_{\text{interaction}} < 0.1$), the combined effect was estimated as follows:

$$RR = \exp\{(\beta_1 \times \text{allergic sensitization}) + (\beta_2 \times \text{FLG variants}) + (\beta_3 \times [\text{allergic sensitization} \times \text{FLG variants}])\}$$

. The term 'combined effect' was used to describe the joint impact of two individual risk factors plus their interaction on the occurrence of the outcome. In all GEE models, sex and age at follow-up were included as potential confounders. To evaluate statistical significance of main effects of covariates (not interaction terms), a P value ≤ 0.05 was used as indicator of statistical significance.

Population attributable fractions (PAFs), defined as the proportion of disease that can be related to a specific risk factor(s) [29], were estimated using the following formula:

$$PAF = p_c \times [(RR - 1) / RR]$$

where p_c is the proportion of diseased-individuals that are exposed [29, 30]. This approach yields valid PAF estimates in the presence of confounding and/or effect modification, only when using adjusted RRs [29, 30]. In case of statistically significant interactions between allergic sensitization and *FLG* variants, the RRs for the combined effect were used to estimate PAFs associated with both risk factors. If no interaction was detected, adjusted RRs were used to estimate separate PAFs for each of the two risk factors and an overall PAF was obtained using the following formula:

$$PAF_{\text{overall}} = 1 - [(1 - PAF_{\text{allergic sensitization}}) \times (1 - PAF_{\text{FLG variants}})] [29].$$

Results

A total of 1,377, 1,210, 1,345, and 1,298 children, out of 1,456, had available information on eczema, asthma, and rhinitis diagnosis at ages 1-or-2, 4, 10, and 18 years, respectively

(Table 1). SPT results were available for 515 (age 1-or-2: only children with any symptoms), 982, 1,036, and 853 children at ages 1-or-2, 4, 10, and 18 years, respectively. The proportion of positive SPTs increased from 20.6% to 41.4% between ages 1-or-2 and 18 years. Although SPT at age 1-or-2 was performed only on symptomatic children, the proportion of sensitized children at ages 1-or-2 and 4 years were similar (20.6% vs. 19.6%). The combined proportion of carriers (heterozygous) of *FLG* variants R501X, 2282del4 and S3247X was 10.3% (118/1150; Table 1).

Prevalence of allergic disorders

The period prevalence of “eczema only” was between 7.9% and 8.5% during the first 10 years of life and decreased to 5.6% at 18 years of age (Table 1). “Asthma only” was most prevalent (10.2%) at 4 years. The period prevalence of “rhinitis only”, despite the drop at age 4, demonstrated an increasing trend over time by reaching 20.4% at 18 years of age. The comorbidity of “eczema and asthma” and “eczema and rhinitis” ranged from 0.6% to 2.4% and 0.6% to 3.6%, respectively, between 1-or-2 and 18 years. Having “asthma and rhinitis” was most common (9.0%) at age 18 years. A coexistence of eczema, asthma, and rhinitis was found in approximately 2% over the study period.

Association analysis: single risk factor models

FLG variants showed increased risk of “eczema only” ($RR = 1.37$, $P = 0.101$) and “rhinitis only” ($RR = 1.27$, $P = 0.064$; Table 2) that was not statistically significance, during the first 18 years of life. However, *FLG* variants predisposed to increased risk of having “eczema + asthma” ($RR = 2.41$, $P < 0.01$), “asthma + rhinitis” ($RR = 1.52$, $P < 0.01$), and a trend for increased risk of “eczema + rhinitis” ($RR = 1.69$, $P = 0.077$). Moreover, the risk of “eczema, asthma, and rhinitis” was 3.43 fold-higher among those with *FLG* haploinsufficiency than those with functional *FLG* gene. Accordingly, *FLG* variants showed stronger associations with allergic comorbidities than single disorders.

Allergic sensitization, statistically significantly, increased the risk of “eczema-”, “asthma-”, and “rhinitis-only” by 1.70-, 2.50-, 2.76-fold over the study period (Table 2). In addition, allergic sensitization predisposed to increased risk of having “eczema + asthma” ($RR = 6.64$, $P < 0.01$), “eczema + rhinitis” ($RR = 3.48$, $P < 0.01$), and “asthma + rhinitis” ($RR = 4.71$, $P < 0.01$). In particular, allergic sensitization was associated with an increased risk for the coexistence of “eczema, asthma, and rhinitis” ($RR = 9.22$, $P < 0.01$).

Association analysis: two risk factors models

Simultaneously adjusting for *FLG* variants and allergic sensitization plus their interaction effect allowed us to determine their individual and combined effects. In these adjusted models, *FLG* variants did not show any association with allergic disorders (Table 3). In contrast, allergic sensitization was significantly associated with all allergic disorders. *FLG* variants and allergic sensitization showed possible interactive effects for three of the studied comorbidities. Combined effect of allergic sensitization and *FLG* variants increased the risk of “eczema and asthma” ($RR = 13.67$, $P < 0.01$), “asthma and rhinitis” ($RR = 7.46$, $P < 0.01$), “eczema, asthma, and rhinitis” ($RR = 23.44$, $P < 0.01$; Table 3). To estimate whether the possible misclassifications of rhinitis/asthma at age 1-or-2 years influenced our results,

we repeated the previous analyses while excluding data from the 1-or-2 years. Comparable results were obtained when we repeated our analysis by only including data from 4, 10, and 18 years follow-ups in the GEE analysis (data not shown).

Sex differences

To determine whether sex is a potential effect modifier, we ran separate models for boys and girls. The effects of *FLG* variants and allergic sensitization on the risk of having single and multiple morbidities were similar for boys and girls. For instance, *FLG* variants associated with 2.95-fold (95% CI: 1.35 – 6.42, $P < 0.01$) higher risk of having ‘eczema + asthma + rhinitis’ among boys; whereas, a 3.97-fold (95% CI: 1.96 – 8.05, $P < 0.01$) higher risk was observed among girls ($P_{\text{interaction}}$ for sex = 0.597; detailed data not shown).

Population attributable fractions (PAFs)

PAFs related to *FLG* variants and allergic sensitizations were estimated for the different allergic disorders using adjusted RRs from Table 3. A larger fraction of allergic disorders in the population can be related to allergic sensitization than to *FLG* variants, when simultaneously adjusting for their effects (Table 4). Around 13.7% to 29.5% of single allergic disorders might be related to having both allergic sensitization and *FLG* variants. High PAFs for having “eczema, asthma, and rhinitis” (49%), “eczema + asthma” (33.7%), and “asthma + rhinitis” (21.2) were related to the combined effect of both allergic sensitization and *FLG* variants.

Discussion

In this well-characterized longitudinal study, allergic comorbidities were prevalent at all ages. For the first time, we assessed the single and combined effects of *FLG* variants and allergic sensitization on the development of single allergic disorders and comorbidities. *FLG* variants, when adjusting for the effect of allergic sensitization, did not show any association with allergic disorders. Against that, allergic sensitization, adjusting for the effect of *FLG* variants, increased the risk of all allergic disorders. Substantial combined effects of both factors were detected for allergic comorbidities. For instance, a 23-fold increased risk for having “eczema, asthma, and rhinitis” (approximately 2% of the cohort) was associated with the presence of both *FLG* variants and allergic sensitization.

Investigating and understanding the role of deviated immune responses, i.e. allergic sensitization, in the atopic diathesis have dominated the research field. However, the discovery of *FLG* variants and their pronounced role in the development of allergic disorders, eczema in particular, has caused a shift in the research paradigm toward understanding the contribution of dysfunctional skin barrier. *FLG* variants are considered to result in a compromised epidermal barrier that is characterized by increased penetration of allergens and microbes and elevated transepidermal water loss [22]. In eczema, the role of *FLG* variants is well established; however, since *FLG* is not expressed in the respiratory epithelia its contribution to the development of allergic disorders of the airways is not clear [22, 31]. A suggested pathway is that cutaneous sensitization, facilitated by *FLG* variants, could lead to local and systematic inflammation at distant organs (i.e., lung and nasal

tissues) [31]. Also the contrary is of importance, since it has been shown that inflammatory Th2 cytokines can also alter the integrity of the skin barrier, even in the absence of genetic variants that disrupt its integrity and function [32]. Hence, the two competing views: “inside-outside” which suggests that immunologic abnormality is the initiating and triggering step in the pathogenesis of allergic disorders and the “outside-inside” which posits that a defective epidermal barrier is the driver with epiphenomenal immunological sequelae, seem to be plausible and to some extent interdependent [33]. Therefore, simultaneously considering the contribution of genetic predisposition toward a defective skin barrier (i.e., *FLG* variants) and the propensity for immune dysregulation (i.e., allergic sensitization) could provide new and improved insights into the etiopathogenesis of allergic disorders.

Previous studies showed that *FLG* variants increased the risk of eczema and predisposed to higher risk of asthma and rhinitis only in the presence of coexisting eczema [34, 35]. Hence, suggesting that *FLG* variants increase the risk of allergic comorbidities, such as ‘asthma plus eczema’ and ‘rhinitis plus eczema’. Our results further support and add to the previously reported findings. However, we showed that *FLG* variants did not pose any increased risk of single and multiple allergic disorders while controlling for the effect of allergic sensitization. But, significant interactive effects of both risk factors were associated with increased risk of allergic comorbidities. Although previous investigations inconsistently showed that *FLG* variants increased the risk of allergic sensitization [35–37], we did only observe a weak association between *FLG* variants and allergic sensitization in this cohort [27]. The possibility of an association between *FLG* variants and allergic sensitization cannot be totally excluded; however, since only around 15% of those with allergic sensitization carry *FLG* variants in our cohort, we speculate that any possible indirect effect of *FLG* variants on allergic diseases that goes through allergic sensitization to be minimal. Moreover, the possibility of non-independence between the two factors, also known as gene-environment correlation, might bias the interactive effects [38]. Due to the weak non-significant association between *FLG* variants and allergic sensitization in our cohort, we have little concern that the unlikely non-independence of the two factors might bias the observed interactive effects. In support of our analytical model, we clearly demonstrated in a previous report that *FLG* variants posed no increased risk of eczema in the absences of allergic sensitization, whereas, allergic sensitization increased the risk of eczema independent of *FLG* variants and the combined effect of both factors led to elevated risk above and beyond their individual effects [27]. Similarly, Wang *et al.* showed that the association between *FLG* variants and eczema was limited to a subset of children with IgE levels ≥ 100 kU/L [39]. Hence, we suggest considering the combined effect of both risk factors rather than taking the cause-effect approach could lead to a better risk assessment.

Our estimated PAFs suggested that approximately 21% to 49% of allergic comorbidities in the population may be attributed to the presence of both *FLG* variants and allergic sensitization. In agreement with results of this report, it has been shown that a large proportion (38%) of allergic comorbidities can be explained by allergic sensitization alone [40]. Although, the contribution of *FLG* variants to the overall PAFs seems to be limited while controlling for the main effect of allergic sensitization, the PAFs associated with the combined effects of both risk factors (i.e., in the presence of possible interactive effects

between *FLG* variants and allergic sensitization) is substantial. Our results suggest that *FLG* variants need allergic sensitization as a necessary component to execute its adverse effect. Therefore, early identification of those who are genetically predisposed to a dysfunctional skin barrier and who have increased allergic sensitization propensity may expedite the success of intervention/prevention efforts and measures.

The etiologic approach taken so far by research in the field of *FLG* variants and allergic disorders has substantially improved our understanding of these multifactorial disorders. However, as suggested by Ballardini *et al.*, allergic disorders should not be studied as single entities [17], rather investigating their coexistence could assist in identifying shared underlying risk factors. Hence, we considered all possible and non-overlapping combinations of eczema, asthma, and rhinitis. Through this approach we were able to show that *FLG* variants increased the risk of allergic comorbidities rather than single disorders, suggesting its contribution towards a more severe and complex clinical phenotype. The effect of *FLG* variants when combined with the effect of allergic sensitization predisposed to even higher risk of allergic comorbidities. In contrast to investigating the coexistence of allergic disorders, evidence supporting the progression of events (i.e., allergic march) also exists [41, 42]. For instance, a valid assumption is that eczema predisposes to allergic sensitization, which in turn predicts asthma and rhinitis. However, evidence supporting both pathways exists: (i) eczema precedes allergic sensitization and (ii) allergic sensitization precedes eczema [43, 44]. In the context of *FLG* variants, we have demonstrated, previously, that the combined effect of *FLG* variants and allergic sensitization predisposed to increased risk of subsequent eczema [27]. Also, we showed that the combination of *FLG* variants and eczema (*FLG* variants alone does not) increased the risk of subsequent allergic sensitization, which was limited to the first 10 years of life. Taken together, these observations suggest that multiple pathways could underlie the development of allergic disorders. Therefore, the development of allergic disorders is more complex and do not necessarily follow the atopic march paradigm. Although our approach does not take into account the sequence of the individual allergic manifestations, results of this report demonstrated that the co-manifestation of allergic disorders is common and *FLG* variants and allergic sensitization are common risk factors.

Major strengths of our study are the repeated phenotyping and the low attrition (ranging from 5% to 17%). *FLG* variants genotypes were available for the majority (80%) of the study population. Previously, we demonstrated that the genotyped sample did not differ from the total cohort with respect to multiple characteristics [27]. Although skin prick tests were performed at the different follow-ups to determine sensitization status, not measuring specific IgE is a limitation to our study. Moreover, since SPTs at ages 1 and 2 years were performed on symptomatic children, we speculate that selection bias leading to underestimated 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 age 4 years with outcomes at the same age (data not shown), since also the reference group had some symptoms. Hence, the estimated RRs at age 1-or-2 might be

biased; however, the extent of the possible selection bias is minimal since our results did not noticeably change when we excluded the 1-or-2 years follow-up data from the analyses. Such observations further demonstrate the robustness of the GEE method in providing population averaged estimates (RRs) while accounting for the within-subject correlations of repeated measurements. Misclassification of eczema cases is minimal since a high proportion of subjects showed typical manifestation of eczema in usual locations (antecubital or popliteal fossae, ankles, face or neck for 97% at 1 year, 91% at 2 years, 75% at 4 years, 86% at 10 years and 76% at 18 years) [7]. In addition, the prevalence of eczema in our cohort (13.7% at age 10 years and 12.3% at age 18 years) is comparable to results from ISAAC studies conducted among children aged 13–14 years in the United Kingdom (UK) (14.7% phase one and 10.6% phase three) and other studies conducted in the UK [45–47]. Other, potential limitations are the definition of asthma and rhinitis symptoms in early life. Our asthma conclusion at ages 10 and 18 years followed the ISAAC criteria [25], which was at that time not available for assessments at ages 1, 2, and 4 years. Although slightly different methods were used to define asthma; however, in a previous report we demonstrated that the minor change in asthma definition did not influence the validity of our asthma classification [48]. 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 (6.5% vs. 2.5% at age 4 years) might have been influenced by misclassifying ‘viral induced infectious rhinitis’ [8]. The influence of the possible misclassification of rhinitis at 1-or-2 years on the overall results of the study is minimal since similar results were obtained when we reran our analyses while excluding the 1-or-2 year data.

In conclusion, comorbidities of eczema, asthma, and rhinitis are prevalent and both allergic sensitization and *FLG* variants are common risk factors that predispose to the comorbidity of allergic disorders. The combination of both risk factors was associated with elevated risk of coexisting allergic manifestations. Approximately 21% to 49% of the comorbidities in the population are related to both allergic sensitization and *FLG* variants. The coexistence of allergic disorders might reflect more severe clinical phenotypes that require more and longer medical care. However, investigations of comorbidities and their underlying etiological risk factors are limited. Therefore, additional etiological investigations regarding allergic comorbidities are recommended, as this will allow for a better phenotypic characterization.

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Table 1

Characteristics of the Isle of Wight study population at different ages

	Follow-up at age			
	1-or-2 years	4 years	10 years	18 years
% (n)	n = 1377	n = 1210	n = 1345	n = 1298
Sex				
Male	50.8 (700)	51.1 (618)	50.7 (682)	49.6 (644)
No allergic disorders	68.9 (949)	74.1 (897)	61.8 (832)	52.7 (684)
Eczema only	8.5 (117)	7.9 (96)	8.0 (107)	5.6 (73)
Asthma only	4.9 (68)	10.2 (123)	6.3 (84)	5.5 (71)
Rhinitis only	6.5 (89)	2.5 (30)	13.4 (181)	20.4 (265)
Eczema + asthma	1.9 (26)	2.4 (29)	1.0 (14)	0.6 (7)
Eczema + rhinitis	1.8 (25)	0.6 (7)	2.4 (32)	3.6 (47)
Asthma + rhinitis	5.5 (75)	1.3 (16)	4.7 (63)	9.0 (117)
Eczema + asthma + rhinitis	2.0 (28)	1.0 (12)	2.4 (32)	2.6 (34)
Allergic sensitization	n = 515	n = 982	n = 1036	n = 853
1 positive SPT	20.6 (106)	19.6 (192)	26.9 (279)	41.4 (353)
<i>FLG</i> variants *	n = 1150			
Yes		10.3 (118)		

SPT: Skin prick test

* *FLG* variants were present in 118 participants of the 1150 who were genotyped. The prevalence of *FLG* variants changes based on the analytical sample.

Table 2

Single risk factor models: associations of *FLG* variants and allergic sensitization with allergic conditions: longitudinal analysis from age 1-or-2 to 18 years

	Single risk factor model: <i>FLG</i> variants*		Single risk factor model: Allergic sensitization*	
	<i>FLG</i> variants genotype		Allergic sensitization	
	WT (k = 3831)[§]	LOF (k = 442)[§]	SPT – (k = 2453)[§]	SPT + (k = 929)[§]
No allergic disorders (Ref.), % (k)	64.2 (2459)	54.3 (240)	65.3 (1602)	29.7 (276)
Eczema only, % (k)	7.6 (291)	8.8 (39)	9.5 (234)	9.8 (91)
RR (95% CI)	1.00	1.37 (0.94–1.99)	1.00	1.70 (1.36–2.12) [‡]
Asthma only, % (k)	6.6 (253)	6.1 (27)	7.2 (177)	9.7 (90)
RR (95% CI)	1.00	1.07 (0.71–1.61)	1.00	2.50 (1.99–3.14) [‡]
Rhinitis only, % (k)	11.2 (428)	12.4 (55)	10.0 (246)	20.8 (193)
RR (95% CI)	1.00	1.27 (0.99–1.64)	1.00	2.76 (2.34–3.27) [‡]
Eczema + asthma, % (k)	1.4 (52)	2.9 (13)	1.1 (27)	4.1 (38)
RR (95% CI)	1.00	2.41 (1.28–4.57) [‡]	1.00	6.64 (4.18–10.54) [‡]
Eczema + rhinitis, % (k)	2.0 (76)	2.7 (12)	2.1 (52)	4.2 (39)
RR (95% CI)	1.00	1.69 (0.95–3.02)	1.00	3.48 (2.35–5.18) [‡]
Asthma + rhinitis, % (k)	5.4 (205)	6.8 (30)	3.7 (90)	15.1 (140)
RR (95% CI)	1.00	1.52 (1.04–2.23) [†]	1.00	4.72 (3.76–5.94) [‡]
Eczema + asthma + rhinitis, % (k)	1.8 (67)	5.9 (26)	1.0 (25)	6.7 (62)
RR (95% CI)	1.00	3.43 (2.02–5.83) [‡]	1.00	9.22 (5.63–15.12) [‡]

WT: Wild-type; LOF: Loss-of-function; SPT –: Negative skin prick test; SPT +: Positive skin prick test; Ref.: represent the common reference category; k: refers to the total number of repeated measurements.

* In all GEE models sex and age at follow-up were included as potential confounders.

[§] The presented numbers in parenthesis (k =) refer to the number of repeated measurements. See Table 1 for more information on the number of individuals.

[†] *P*-value < 0.05

[‡] *P*-value < 0.01

Two risk factors model: associations of *FLG* variants and allergic sensitization with allergic conditions: longitudinal analysis from age 1-or-2 to 18 years

Table 3

	Two risk factors model: <i>FLG</i> variants and allergic sensitization*				
	<i>FLG</i> variants genotype		Allergic sensitization		RR _{Interaction} (<i>P</i> _{Interaction}) [#] Combined effect [‡]
	WT (k = 2707) [§]	LOF (k = 328) [§]	SPT – (k = 2164) [§]	SPT + (k = 871) [§]	
No allergic disorders (Ref.), % (k)	56.9 (1540)	46.3 (152)	66.1 (1431)	30.0 (261)	
Eczema only, % (k)	9.3 (252)	10.1 (33)	9.2 (200)	9.8 (85)	0.98
RR (95% CI)	1.00	1.11 (0.81–1.54)	1.00	1.74 (1.38–2.19) [‡]	(0.961)
Asthma only, % (k)	7.6 (205)	6.4 (21)	6.7 (144)	9.4 (82)	1.05
RR (95% CI)	1.00	0.99 (0.65–1.53)	1.00	2.65 (2.07–3.39) [‡]	(0.926)
Rhinitis only, % (k)	13.3 (360)	14.6 (48)	10.4 (226)	20.9 (182)	0.85
RR (95% CI)	1.00	1.21 (0.95–1.55)	1.00	2.68 (2.25–3.19) [‡]	(0.538)
Eczema + asthma, % (k)	1.6 (44)	4.0 (13)	1.0 (22)	4.0 (35)	5.46
RR (95% CI)	1.00	0.49 (0.08–3.03)	1.00	5.12 (2.95–8.90) [‡]	(0.016)
Eczema + rhinitis, % (k)	2.5 (68)	3.4 (11)	2.1 (46)	3.8 (33)	1.48
RR (95% CI)	1.00	1.52 (0.85–2.69)	1.00	3.19 (2.09–4.89) [‡]	(0.511)
Asthma + rhinitis, % (k)	6.7 (182)	8.2 (27)	3.4 (73)	15.6 (136)	2.04
RR (95% CI)	1.00	0.79 (0.34–1.89)	1.00	4.57 (3.52–5.93) [‡]	(0.070)
Eczema + asthma + rhinitis, % (k)	2.1 (56)	7.0 (23)	1.0 (22)	6.5 (57)	3.59
RR (95% CI)	1.00	1.02 (0.25–4.16)	1.00	6.43 (3.62–11.42) [‡]	(0.053)
					23.44 (12.27–44.78) [‡]

WT: Wild-type; LOF: Loss-of-function; SPT –: Negative skin prick test; SPT +: Positive skin prick test; Ref.: represent the common reference category; k: refers to the total number of repeated measurements; RR_{Interaction}: Risk ratio associated with the interaction term.

* In all GEE models sex and age at follow-up were included as potential confounders.

[#] *P*-value < 0.1 was used to indicate presence of possible statistical interaction. If there was no evidence of interaction, the interaction term was removed from the regression model and only the main effects were modeled.

^{\$} The presented numbers in parenthesis (k =) refer to the number of repeated measurements. See Table 1 for more information on the number of individuals.

[†] Combined effect was estimated only in the presence of possibly statistically significant interaction term (*FLG* variants $\times$ allergic sensitization; $P_{\text{Interaction}} < 0.1$).

[‡] P -value < 0.01 .

Table 4

Population attributable fractions of different allergic conditions related to *FLG* variants and allergic sensitization

	<i>FLG</i> variants	Allergic sensitization	Overall PAF [†] (%)
	PAF (%) [*]	PAF (%) [*]	
Eczema only	1.2	12.7	13.7
Asthma only	n/a [‡]	22.6	22.6 [¶]
Rhinitis only	2.1	28.0	29.5
Eczema + asthma	n/a ^{\$}	n/a ^{\$}	33.7
Eczema + rhinitis	4.8	28.7	32.1
Asthma + rhinitis	n/a ^{\$}	n/a ^{\$}	21.2
Eczema + asthma + rhinitis	n/a ^{\$}	n/a ^{\$}	49.0

PAF: population attributable fraction; n/a: not available

^{*} PAFs were estimated using adjusted risk ratios obtained from Table 3.

[†] In the presence of statistically significant interaction, overall PAF were estimated using the RR for the combined effect obtained from Table 3.

[‡] PAF was not estimated due to RR < 1.00.

^{\$} PAF was not estimated due to the presence of possible statistical interaction (see Table 3).

[¶] This value represents the PAF associated with allergic sensitization only since PAF associated with *FLG* variants was not estimable.