The burden of disease associated with filaggrin mutations: A population-based, longitudinal birth cohort study

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Background: Atopic disease is a major health problem. Mutations in the filaggrin gene *(FLG)* confer major susceptibility to eczema and related asthma.

Objective: We sought to determine the natural history and burden of atopic disease conferred by the 2 most common *FLG* mutations in a large, population-based birth cohort study.

Methods: We analyzed the effect of the most common null alleles (R501X and 2282del4) on several atopic phenotypes in a cohort of approximately 7000 English children born in 1990-1991.

Results: *FLG* null alleles associated strongly with eczema; eczema associated with these mutations presents in early life and is more persistent (hazard ratio for eczema resolution for those with *FLG* mutations to *FLG* wild type, 0.67; 95% CI, 0.58-0.77; $P = 5 \times 10^{-8}$). *FLG* mutations conferred a population asthma risk of 1.80 (95% CI, 1.34-2.41; $P = .00019$); asthma risk was especially high in the context of eczema (odds ratio, 3.16; 95% CI, 2.25-4.43; $P = 1.4 \times 10^{-11}$). Strong associations were identified with sensitization to grass, house dust mite, and cat dander and sensitization to multiple allergens (odds ratio, 2.12; 95% CI, 1.03-4.37; $P = 5.42 \times 10^{-27}$).

Conclusion: *FLG* mutations are strong genetic determinants of eczema, early wheeze, asthma in the context of eczema, and atopic sensitization. They confer risk of a particular trajectory for eczema, with increased duration of disease and greater risk of asthma and multiple allergic sensitizations. *FLG* alleles help define the risk profile of children with eczema and help define the "eczema plus early wheeze" and "eczema plus asthma" phenotypes. (J Allergy Clin Immunol 2008;121:872-7.)

Key words: *Eczema, atopic dermatitis, asthma, skin barrier, filaggrin, birth cohort, atopy*

Atopic diseases, such as atopic dermatitis (AD; eczema), asthma, food allergies, and hay fever, are among the most common diseases of children in developed societies. The interrelatedness of various manifestations of the atopic diathesis has been well documented. A strong inherited component is a feature of these conditions, with evidence of a shared genetic basis. Recently, we, and subsequently others, have shown that loss-of-function mutations in gene encoding the skin barrier protein filaggrin confer major susceptibility to AD and related asthma.[1-4] Filaggrin is a key barrier protein that has a role in the compaction of keratin filaments in the stratum corneum and thus contributes to barrier formation and has an additional role in regulating stratum corneum hydration. Although loss-of-function mutations in the filaggrin gene *(FLG)* have been shown to strongly predispose to eczema and the genetic architecture is complex, with up to 6 recurrent mutations in the European population and several other family-specific mutations,[5] 2 mutations (R501X and 2282del4) are the most significant in the United Kingdom population.[5] To observe the effect of these 2 most prevalent *FLG* mutations on atopic outcomes on a population basis, we studied a large, well-characterized, population-based birth cohort (the Avon Longitudinal Study of Parents and Children [ALSPAC] cohort). This resource enables detailed analysis of the effects of the common *FLG* mutations on a range of atopic phenotypes, is a particularly powerful tool to examine the risk of *FLG* mutations on a nonselective basis, and facilitates the examination of the relationship between susceptibility to eczema, asthma, and additional sensitization outcomes.

METHODS

Participants

The ALSPAC is a longitudinal, population-based birth cohort study that recruited 14,541 pregnant women residing in Avon, United Kingdom, with expected dates of delivery between April 1, 1991, and December 31, 1992. There were 14,062 liveborn children. The study protocol has been described previously,[6,7] and further details are available on the ALSPAC Web site

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Abbreviations used
ALSPAC: Avon Longitudinal Study of Parents and Children
AD: Atopic dermatitis
FLG: Filaggrin gene
OR: Odds ratio
PAR: Population attributable risk

(http://www.alspac.bris.ac.uk). Ethical approval for all aspects of data collection was obtained from the ALSPAC Law and Ethics Committee (institutional review board 00003312).

Data collection

The extant nosology of atopic disease is confusing, and terms such as *eczema*, *flexural dermatitis*, and *atopic dermatitis* are frequently used interchangeably in the literature. Recently, a World Allergy Organization report suggested the use of *eczema* as preferable to *atopic dermatitis*,[8] and in this article we generally follow this recommendation and use the term *eczema*. In following this definition, we recognize that many previous genetic studies on these conditions have used definitions of AD, and in fact, we have previously used a definition of AD on this very cohort. Because this study was designed before the establishment of the UK Working Party diagnostic guidelines for AD,[9] in previous studies we defined individuals with eczema as those with reports of flexural dermatitis at 2 time points between 6 and 42 months,[10] and in this article we retain this diagnostic definition of eczema for internal consistency between articles on this cohort.

At 6, 18, 30, 42, 57, and 81 months of age, mothers were asked whether their children had skin rashes in the joints or creases of the body. Children attended research clinics annually from the ages of 7 to 11 years and at these clinics the presence of flexural dermatitis was noted according to the International Study of Asthma and Allergies in Childhood protocol.[11] Clinician-trained observers noted the presence of any flexural dermatitis of greater than 3 cm in diameter in any of the following areas: around the eyes, the sides, or front of the neck; in front of the elbows; behind the knees; or in front of the ankles.

At 6, 18, 30, 42, 54, 69, and 81 months after birth, study mothers were sent a self-completion questionnaire about the health of their children. They were asked to report the occurrence of 15 common symptoms, including wheezing, in the previous 12 months (6 months for the initial questionnaire) and, if present, whether they consulted a doctor. In a separate section they were asked whether in the past 12 months (6 months in the first questionnaire) their child had "[w]heezing with whistling on the chest when (s) he breathed?" *Early wheeze* was defined as a positive answer to these questions at any time point up to and including the 42-month questionnaire. Asthma was defined on the basis of 2 questions asked when the children were 91 months of age: (1) whether a doctor had ever diagnosed the child as having asthma and (2) whether the child had "wheeze with whistling" in the past 12 months. Positive responses to both these questions identified children with asthma. We divided both asthma and eczema into atopic (based on positive skin prick test responses of $\geq$1 mm wheal for grass, house dust mite, or cat) and nonatopic variants. Children's atopic status was determined at age 7 to 8 years on the basis of skin prick test responses to common allergens, including house dust mite *(Dermatophagoides pteronyssinus)*, mixed grasses, and cat (ALK-Abelló, Hørsholm, Denmark). A positive response was defined as a mean wheal diameter of 3 mm or greater. Bronchial responsiveness to methacholine at age 8 years was measured by using the method of Yan et al[12] and expressed for each subject as the dose-response slope of FEV_1 (percent of baseline) per micromole of methacholine.[13] These results are notated such that a greater number indicates increased responsiveness.

At 54 months of age, the mothers were asked whether their children had any allergy to pollen. At 81 months, more detailed questions were asked to determine hay fever prevalence. Mothers were asked the following: "In the past 12 months, has your child had a problem with sneezing, runny or blocked nose when he/she did not have a cold or the flu?" Mothers were also asked to indicate in which months these symptoms were experienced. Hay fever was defined as a positive response to the first question experience between May and September with no report between October and April.

Genotyping

The *FLG* variants R501X and 2282del4 were initially genotyped with the TaqMan allelic discrimination assays (Applied Biosystems, Foster City, Calif) in 384-well plates by using the previously reported primers, probes, and reaction conditions.[1,5] A substantial fraction of the samples, including all the identified homozygotes of both variants and all 2282del4 carriers, were double-checked by means of restriction enzyme digestion (R501X) or fluorescent-labeled PCR methodology (2282del4), as previously described.[1] The final dataset showed complete concordance across the various analysis methods. There was no significant deviation from Hardy-Weinberg equilibrium (Table I).

Confounder effects

We examined associations between *FLG* mutations in the children and the following potential confounders: sex, maternal smoking in pregnancy, environmental tobacco exposure in childhood, maternal asthma, maternal eczema, maternal hay fever, maternal social class, parity, breast-feeding, exposure to central or storage heating, and maternal age.

Statistical methods

The prevalence of *FLG* mutations in those children with each outcome of interest is presented. Logistic regression was performed to obtain odds ratios (ORs) and 95% CIs for each outcome according to *FLG* mutations. For bronchial responsiveness, mean differences in the dose-response slope of FEV_1 were calculated according to *FLG* mutation. Stratified analysis was performed to assess the effects of *FLG* mutations on associated atopic outcomes in children with and without a history of rash (any rash reported up to and including 81 months). Multinomial logistic regression was also performed to test for differences between the associations with various phenotypes and *FLG* alleles in the presence or absence of eczema. Population attributable risks (PARs) were calculated as follows: $(p\,[r-1])/(p\,[r-1]+1)\times 100$, where p is the proportion of the population with the exposure and r is the risk (OR) in the population associated with that exposure. Survival analysis was performed to determine the persistence of rash report throughout childhood by using questionnaire data up to 81 months and the presence of flexural dermatitis as recorded at the annual clinics from 7 to 11 years of age. Kaplan-Meier plots are presented, and Cox proportional hazards were calculated and stratified by *FLG* mutation status. All analyses were performed with SPSS for Windows v.12.0.1 (SPSS, Inc, Chicago, Ill) or Intercooled STATA for Macintosh V8.0 (StataCorp, College Station, Tex).

RESULTS

Each of these 2 mutations is known to have essentially identical biochemical consequences,[1,5] and in this study association scores and penetrance results for the primary phenotypes discussed are very similar for these 2 mutations (see Tables E1 and E2 in the Online Repository at www.jacionline.org); we present and analyze here the combined genotypic data. A summary of genotyping results along with a phenotypic profile of the cohort is given in Table I.

FLG null alleles strongly predispose to eczema at all ages

FLG null alleles were associated with flexural dermatitis at all 6 questionnaire time points and at all 3 observed time points (see Table E3 in the Online Repository at www.jacionline.org). Strong associations were also seen with eczema, and all of the homozygotes/compound heterozygotes (6/6) for whom full data were available had

TABLE I. Population characteristics in those who were genotyped (n = 6971)

Genotype/population characteristic	Percentage with characteristic
FLG null allele	
AA	91.2% (6361/6971)
Aa	8.7% (604/6971)
aa	0.1% (6/6971)
Rash reported	
6 mo	17.8% (1134/6386)
18 mo	27.6% (1744/6313)
30 mo	26.8% (1611/6007)
42 mo	19.6% (1349/5904)
57 mo	24.0% (1359/5663)
81 mo	19.6% (1053/5361)
Flexural dermatitis observed	
7 y	7.3% (397/5404)
9 y	7.6% (378/4999)
11 y	5.7% (263/4336)
Eczema	
Overall	27.5% (1445/5255)
Atopic	4.1% (193/4764)
Nonatopic	18.1% (863/4764)
Asthma	
Overall	8.5% (447/5231)
Atopic	2.6% (107/4123)
Nonatopic	5.9% (243/4123)
Reaction to skin prick tests	
Grass	8.3% (401/4809)
Cat	4.6% (220/4784)
DP	7.2% (346/4821)
Hay fever	
54 mo	2.4% (142/5833)
81 mo	5.1% (275/5395)
11 y	16.8% (779/4628)
Male sex	51.9% (3618/6971)

AA, Wild type for both mutations; *Aa*, heterozygote for either mutation; *aa*, either a homozygote for each individual mutation (ie, R501X/R501X or 2282del4/2282del4) or a compound heterozygote for each mutation (ie, 2282del4/R501X); *DP*, *Dermatophagoides pteronyssinus*.

eczema (Table II). Although associations were very strong for both atopic and nonatopic eczema, the association was notably stronger, with higher ORs for atopic eczema than for nonatopic eczema ($P = .0023$ for test between the effect estimates, Table II).

FLG alleles are associated with persistent eczema

To examine the effect of *FLG* mutations on the disease trajectory and persistence of eczema, we performed a survival analysis based on the subcohort of children with eczema established by 42 months. Lack of persistence (ie, clearance) beyond 42 months was determined by the first date of absence of report of rash that was not followed by a subsequent report of flexural rash (Fig 1). In children with eczema, the mean time that eczema persisted for those with *FLG* mutations was 76.7 months (SD, 29.6) compared with a mean persistence time of 65.6 months (SD, 28.9) for those without *FLG* mutations. In a survival analysis in which we followed the individuals with eczema until they presented without eczema (and with no subsequent eczema), the hazard ratio of individuals bearing *FLG* mutations to those with wild-type *FLG* was 0.67 (95% CI, 0.58-0.77; $P = 5 \times 10^{-8}$), providing strong evidence that the rate of children "growing out" of their AD is much lower among individuals with *FLG* mutations (Fig 1).

FLG mutations strongly predispose to early wheeze and asthma but only in the context of prior eczema

In this study cohort 447 (8.5%) children were given diagnoses of asthma; of those with skin test data, 191 were classified as having atopic asthma and 150 as having nonatopic asthma, as previously defined. *FLG* null alleles strongly predispose to asthma (OR, 1.80, 95% CI, 1.34-2.41; $P = .00019$; Table II). This association was stronger for atopic than nonatopic asthma. Importantly, in the absence of eczema, *FLG* alleles had no association with asthma (OR, 0.80; 95% CI, 0.46-1.41). *FLG* alleles were strongly associated with the "asthma plus eczema" phenotype (OR, 3.42; 95% CI, 2.38-4.90; $P = 1.95 \times 10^{-12}$; $P = .000016$ for test between the effect estimates; Table II). We also examined associations with early wheeze in the presence and absence of eczema. In this analysis it was striking that there was only a weak association with early wheeze in the absence of eczema (OR, 1.24; 95% CI, 0.96-1.61; Table III), whereas a strong association was seen for early wheeze in the presence of eczema (OR, 3.01; 95% CI, 2.31-3.93; $P = 1.81 \times 10^{-18}$; $P = 9.4 \times 10^{-7}$ for test between the effect estimates). There was also a strong association of *FLG* alleles with eczema in the absence of early wheeze (OR, 2.38; 95% CI, 1.80-3.14; $P = 2.37 \times 10^{-11}$; Table III).

As an additional objective measure of airway function, we measured bronchial responsiveness. Subjects heterozygous for R501X had increased bronchial responsiveness (0.44% decrease in FEV_1 per micromole of methacholine; $F = 9.29$, $P = .002$) compared with that seen in wild-type homozygotes. In a combined analysis subjects who were heterozygous for one of the deficiency alleles had increased bronchial responsiveness (0.29% FEV_1 per micromole of methacholine; $F = 4.58$, $P = .01$; Table II), but the combination of homozygotes and compound heterozygotes did not support a linear effect on bronchial responsiveness per deficiency allele carried. However, this group with bronchial responsiveness data available comprised only 3 subjects, as reflected in the wide CIs, and therefore this result should be interpreted with caution.

FLG mutations strongly predispose to atopic sensitization to common allergens

The association with atopic sensitization was stronger for multiply sensitized individuals, with the strongest association noted for those sensitized to all 3 allergens (OR, 2.12; 95% CI, 1.03-4.37; $P = 5.42 \times 10^{-27}$; Table IV). These associations were not evident in the absence of prior eczema (data not shown).

FLG mutations and hay fever

Weaker positive associations were seen for hay fever at all 3 ages examined (Table IV); again, this was not evident in the absence of prior eczema (data not shown).

PARs of *FLG* null alleles and atopic phenotypes

We additionally calculated PARs for several of the key phenotypes studied (Table V). Consistent with the association studies in this cohort, we demonstrate a very high PAR for atopic eczema (15.1%), eczema plus asthma (15.5%), and eczema plus early wheeze (16.6%).

Gene-environment interaction

We tested for potential interaction between *FLG* genotype and the environmental factors of maternal smoking, child exposure to

TABLE II. Association of *FLG* null alleles with eczema, asthma, and bronchial responsiveness

	AA	Aa	aa
Eczema (n = 5255)			
$\chi^2 = 100.9$ ($P = 1.19 \times 10^{-22}$)	1231/4796	208/453	6/6
OR (95% CI)	1.00	2.46 (2.02-2.99)	∞ (4.52-∞)
Atopic eczema (n = 4764)			
$\chi^2 = 89.35$ ($P = 3.96 \times 10^{-20}$)	153/4360	36/398	4/6
OR (95% CI)	1.00	2.73 (1.87-3.99)	4.98 (9.99-382.5)
Nonatopic eczema (n = 4764)			
$\chi^2 = 27.60$ ($P = 1.10 \times 10^{-6}$)	751/4360	110/398	2/6
OR (95% CI)	1.00	1.84 (1.45-2.32)	2.40 (0.44-13.14)
Overall asthma (n = 5231)			
$\chi^2 = 17.1$ ($P = .00019$)	387/4794	59/433	1/64
OR (95% CI)	1.00	1.80 (1.34-2.41)	3.80 (0.39-36.58)
Atopic asthma (n = 4123)			
$\chi^2 = 15.23$ ($P = .0005$)	90/3788	16/331	1/4
OR (95% CI)	1.00	2.09 (1.21-3.60)	13.70 (1.41-132.9)
Nonatopic asthma (n = 4123)			
$\chi^2 = 2.73$ ($P = .255$)	217/3788	26/331	0/4
OR (95% CI)	1.00	1.40 (0.92-2.14)	0 (0-24.48)
Asthma, no eczema (n = 4994)			
$\chi^2 = 0.82$ ($P = .664$)	179/4116	13/374	0/4
OR (95% CI)	1.00	0.79 (0.45-1.41)	0 (0-22.22)
Asthma + eczema (n = 4994)			
$\chi^2 = 53.93$ ($P = 1.95 \times 10^{-12}$)	147/4116	42/374	1/4
OR (95% CI)	1.00	3.42 (2.38-4.90)	9.00 (0.93-87.04)
Bronchial responsiveness at 8 y (n = 3106)			
F = 4.58 ($P = .009$)			
Mean difference in slope (95% CI)	0.00	0.29 (0.09-0.50)	−1.13 (−2.98 to 0.71)

AA, Wild type for both mutations; *Aa*, heterozygote for either mutation; *aa*, either a homozygote for each individual mutation (ie, R501X/R501X or 2282del4/2282del4) or a compound heterozygote for each mutation (ie, 2282del4/R501X).
n = Number of cohort for whom all phenotypic and genotypic data are available for each specific phenotypic outcome (eg, data are complete for bronchial hyperresponsiveness for 3106 subjects). For phenotypes (eg, asthma) dichotomized on the basis of additional data (eg, atopic asthma), the total for the secondary phenotypes is not exactly equal to that of the primary phenotype because not all of the primary phenotypes will have the additional dichotomizing data completed.

environmental tobacco smoke, maternal asthma, maternal eczema, parity, and breast-feeding for the 4 major outcomes of eczema, asthma, eczema plus eczema, and allergic sensitization. We saw no interactions leading to a difference in outcomes for these 4 major atopic phenotypes for the environmental factors tested (see Tables E3-E7 in the Online Repository at www.jacionline.org).

Potential confounders

With the exception of maternal eczema, which was expected, we saw no association between *FLG* mutations and the potential confounders of sex, maternal smoking in pregnancy, the child's environmental tobacco exposure, maternal social class, parity, breast-feeding, central heating, and maternal age (see Table E8 in the Online Repository at www.jacionline.org).

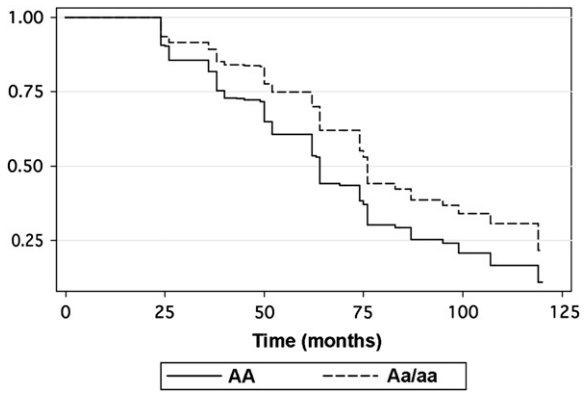

FIG 1. Persistence of eczema: time from first report of rash (of ≥2) to first negative report of rash that was not followed by a positive report (hazard ratio, 0.670; 95% CI, 0.58-0.77; $P = 5 \times 10^{-8}$). Numbers available for analysis at each time point are listed in Table E9 in the Online Repository at www.jacionline.org.

DISCUSSION

Filaggrin is a highly abundant protein in the outermost layers of the epidermis, where it performs a critical cell-compaction process during the formation of the stratum corneum, the terminally differentiated cell layers responsible for skin barrier function.[14] Decreased or absent *FLG* expression because of inheritance of loss-of-function mutations, which are surprisingly prevalent in white populations of European origin, leads to impaired barrier function, allowing the entry of antigens, allergens, and irritants from the environment, which is manifest as atopic disease.[1,15] Although the association between the most common *FLG* variants and eczema is one of the most robustly replicated genotype-phenotype linkages in complex trait genetics, with several case/control association studies and transmission studies reported to date, a large population-based longitudinal study was lacking. Longitudinal data are required to accurately determine disease trajectory, PAR factors, and interrelatedness of phenotypes.

TABLE III. Association of *FLG* genotype with eczema and early wheeze

	AA	Aa	aa
Eczema, no early wheeze (n = 3043)			
$\chi^2 = 48.93$ ($P = 2.37 \times 10^{-11}$)	629/2809	94/231	3/3
OR (95% CI)	1.00	2.38 (1.80-3.14)	∞ (2.70-∞)
Eczema plus early wheeze (n = 3039)			
$\chi^2 = 81.71$ ($P = 1.81 \times 10^{-18}$)	602/2782	114/254	3/3
OR (95% CI)	1.00	3.01 (2.31-3.93)	∞ (2.82-∞)
Early wheeze, no eczema (n = 3810)			
$\chi^2 = 2.63$ ($P = .105$)	1385/3565	108/245	0 (there are no children with no eczema who are aa)
OR (95% CI)	1.00	1.24 (0.96-1.61)	

AA, Wild type for both mutations; *Aa*, heterozygote for either mutation; *aa*, either a homozygote for each individual mutation (ie, R501X/R501X or 2282del4/2282del4) or a compound heterozygote for each mutation (ie, 2282del4/R501X).
n = Number of cohort for whom all phenotypic and genotypic data are available for each specific phenotypic outcome. For phenotypes (eg, eczema) dichotomized on the basis of additional data (eg, plus early wheeze), the total for the secondary phenotypes is not exactly equal to that of the primary phenotype because not all of the primary phenotypes will have the additional dichotomizing data completed.

TABLE IV. Association of *FLG* null alleles with atopic sensitization and hay fever

	Overall population	AA	Aa	aa
Reaction to grass (n = 4809)				
$\chi^2 = 32.5$ ($P = 9.55 \times 10^{-9}$)	8.3%	351/4401	46/402	4/6
OR (95% CI)		1.00	1.49 (1.08-2.07)	23.08 (4.21-126.43)
Reaction to cat (n = 4784)				
$\chi^2 = 36.9$ ($P = 9.55 \times 10^{-9}$)	4.6%	187/4378	30/400	3/6
OR (95% CI)		1.00	1.82 (1.22-2.71)	22.41 (4.49-111.79)
Reaction to house dust mite (n = 4821)				
$\chi^2 = 21.6$ ($P = 2.00 \times 10^{-5}$)	7.2%	303/4411	40/404	3/6
OR (95% CI)		1.00	1.49 (1.05-2.11)	13.56 (2.73-67.46)
Reaction to all 3 allergens (n = 4764)				
$\chi^2 = 121.0$ ($P = 5.42 \times 10^{-27}$)	1.2%	47/4360	9/398	3/6
OR (95% CI)		1.00	2.12 (1.03-4.37)	91.78 (18.05-466.5)
Hay fever at 54 mo (n = 5833)				
$\chi^2 = 9.21$ ($P = .010$)	2.4%	122/5319	19/508	1/6
OR (95% CI)		1.00	1.66 (1.01-2.71)	8.52 (0.99-73.47)
Hay fever at 81 mo (n = 5358)				
$\chi^2 = 4.27$ ($P = .010$)	8.9%	425/4894	51/460	1/4
OR (95% CI)		1.00	1.31 (0.96-1.78)	3.51 (0.36-33.77)
Hay fever at 11 y (n = 4628)				
$\chi^2 = 6.86$ ($P = .032$)	16.8%	700/4231	76/391	3/6
OR (95% CI)		1.00	1.22 (0.94-1.58)	5.04 (1.02-25.04)

AA, Wild type for both mutations; *Aa*, heterozygote for either mutation; *aa*, either a homozygote for each individual mutation (ie, R501X/R501X or 2282del4/2282del4) or a compound heterozygote for each mutation (ie, 2282del4/R501X).
n = Number of cohort for whom all phenotypic and genotypic data are available for each specific phenotypic outcome.

Here we describe a very large, longitudinal, population-based birth cohort study of the comprehensive effects of *FLG* mutations on a wide range of atopic phenotypes. Previously established associations between *FLG* mutations and eczema and asthma have been robustly confirmed in this study. A striking additional association with sensitization to 3 common allergens is described, as well as a weaker positive association with hay fever. Although a large number of outcomes have been considered in these analyses, each was based on a prior hypothesis regarding exposure and outcome. Additionally, many of the outcomes were likely to be highly correlated, making adjustment of multiple testing on the basis of independent outcome variables overly conservative. Therefore we preferred to present the magnitude and precision of effect estimates for each outcome in the results.

We have shown that *FLG* null alleles are strongly associated with childhood asthma and early wheeze, with a notable OR of 1.8 for childhood asthma. In particular, the association with eczema and asthma is strongest for the complex phenotypes of eczema plus early wheeze (OR, 3.01) and eczema plus asthma (OR, 3.16). The association with asthma and wheeze is limited to these phenotypes occurring in the context of pre-existing eczema, and thus these alleles suggest a molecular mechanistic basis to differentiate childhood asthma into eczema plus asthma and asthma without eczema subtypes. These associations strongly replicate earlier studies by us[1] and others[2] that show an association with asthma limited to those with prior eczema. Our data concur with earlier work showing the strongest association with the asthma plus eczema phenotype.[2] In addition, these data substantiate and support recent epidemiologic studies that suggest that children with eczema and wheeze might have a distinct phenotype rather than a progression of related diseases.[16] The mechanisms through which defects in filaggrin, a protein important for skin barrier formation that is not expressed in the bronchial epithelium, contribute to airway disease require further elucidation.

TABLE V. PARs of eczema and asthma phenotypes

	Combined genotype (Aa or aa; prevalence = 0.088)
Atopic eczema	15.1%
Nonatopic eczema	6.9%
Atopic asthma	9.6%
Nonatopic asthma	3.3%
Eczema plus asthma	15.5%
Eczema plus early wheeze	16.6%

Aa, Heterozygote for either mutation; *aa*, either a homozygote for each individual mutation (ie, R501X/R501X or 2282del4/2282del4) or a compound heterozygote for each mutation (ie, 2282del4/R501X).

The systemic effects of eczema could influence airways responsiveness, such that eczema is on the causal pathway between the *FLG* mutations and asthma. Alternatively, *FLG* mutations could predispose to the incidence and severity of eczema in a way that reduces the barrier function of skin to external environmental influences on asthma risk. In the latter case interactions between the exposure and *FLG* genotype could provide evidence as to the causal nature of the environmental exposure responsible for increasing the risk of asthma.[17] In this study we show, importantly, that the risk of asthma and indeed rhinitis is limited to those with prior eczema and is not restricted to those with atopic sensitization. Thus the mechanistic association of eczema and later asthma cannot be reduced solely to sensitization, although a general effect of early eczema on priming a T_H2 phenotype that has effects in later childhood remains one possible mechanism.

Although the molecular pathways mediating this relationship require elucidation, recent experimental work implicates the T_H17 response as a key player between epicutaneous antigen exposure and subsequent airway inflammation.[18]

In this longitudinal cohort we were able to study the trajectory of eczema associated with *FLG* null alleles. Here we clearly demonstrate that children with eczema who carry one of these alleles are more likely to have a more persistent course of disease. In addition, they are more likely to have atopic sensitizations, especially multiple atopic sensitizations. Examination of the genetic and environmental factors that determine sensitization or protection from sensitization in the $FLG^{+/-}$ individuals is a key future research question. Experimental evidence for an association between epicutaneous allergen exposure and a subsequent T_H2 response has been previously reported.[19]

One important implication of this work might be for intervention studies based on early identification of patients with eczema at risk of a more persistent and more atopic disease spectrum. The potential therapeutic implications for early intervention studies in *FLG*-haploinsufficient children with eczema will require further study, but it is possible that early intervention in these children with eczema could alter the trajectory of the disease and the risk of secondary atopic outcomes.[20] This work suggests that substratification of eczema based on *FLG* status could identify those most likely to benefit from such early intervention. The cumulative health burden of these prevalent mutations in a population-based setting is disclosed for the first time. This work places the *FLG* gene as one of the strongest genetic factors for atopic disease. By contributing risk to several facets of the atopic phenotype, *FLG* suggests a mechanistic explanation for the widely observed and acknowledged cosegregation of these complex diseases. The concept of epithelial- or, more specifically, filaggrin-related atopy might have validity and will provide a focus for further studies.

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Clinical implications: *FLG* mutations confer strong susceptibility to eczema and related asthma. Patients with *FLG*-related eczema have more persistent disease and a greater risk of asthma and allergic sensitizations.

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TABLE E1. Association of R501x alleles with eczema, asthma, and sensitization to skin prick tests

	AA	Aa
Eczema (n = 5255)		
$\chi^2 = 46.89$ ($P = 7.50 \times 10^{-12}$)	1339/5032	106/223
OR (95% CI)	1.00	2.50 (1.91-3.27)
Asthma (n = 5231)		
$\chi^2 = 10.04$ ($P = .002$)	415/5008	32/223
OR (95% CI)	1.00	1.85 (1.26-2.73)
Asthma + eczema (n = 4994)		
$\chi^2 = 26.48$ ($P = 2.67 \times 10^{-7}$)	168/4304	22/190
OR (95% CI)	1.00	3.22 (2.01-5.16)
Reaction to any skin prick test (n = 4764)		
$\chi^2 = 12.61$ ($P = .00038$)	429/4564	34/200
OR (95% CI)	1.00	1.97 (1.35-2.89)

n = Number of cohort for whom all phenotypic and genotypic data are available for each specific phenotypic outcome. For phenotypes (eg, asthma) dichotomized on the basis of additional data (eg, atopic asthma), the total for the secondary phenotypes is not exactly equal to that of the primary phenotype because not all of the primary phenotypes will have the additional dichotomizing data completed. Reaction to any skin prick test refers to the reaction to any of grass, cat, or house dust mite (wheal $\geq$3 mm).

TABLE E2. Association of Del4 alleles with eczema, asthma, and sensitization to skin prick tests

	AA	Aa	aa
Eczema (n = 5255)			
$\chi^2 = 49.15$ ($P = 2.13 \times 10^{-11}$)	1334/5016	110/238	2/2
OR (95% CI)	1.00	2.37 (1.83-3.08)	∞ (3.12-∞)
Asthma (n = 5233)			
$\chi^2 = 6.97$ ($P = .031$)	418/5016	29/216	0/2
OR (95% CI)	1.00	1.71 (1.14-2.25)	0 (0-19.89)
Asthma + eczema (n = 4994)			
$\chi^2 = 26.51$ ($P = 1.75 \times 10^{-6}$)	168/4304	22/190	0/2
OR (95% CI)	1.00	3.22 (2.01-5.16)	0 (0-12.56)
Reaction to any skin prick test (n = 4764)			
$\chi^2 = 22.27$ ($P = 1.46 \times 10^{-5}$)	434/4558	28/206	2/2
OR (95% CI)	1.00	1.50 (0.99-2.25)	∞ (5.27-∞)

n = Number of cohort for whom all phenotypic and genotypic data are available for each specific phenotypic outcome. For phenotypes (eg, asthma) dichotomized on the basis of additional data (eg, atopic asthma), the total for the secondary phenotypes is not exactly equal to that of the primary phenotype because not all of the primary phenotypes will have the additional dichotomizing data completed. Reaction to any skin prick test refers to reaction to any of grass, cat, or house dust mite (wheal ≥3 mm).

TABLE E3. Risk of reported flexural rash and observed flexural dermatitis at various time points

	Population prevalence	AA	Aa	aa
Rash at 6 mo (n = 6386)				
$\chi^2 = 59.70$ ($P = 1.09 \times 10^{-13}$)	17.8%	973/5834	157/546	4/6
OR (95% CI)		1.00	2.02 (1.66-2.46)	9.99 (1.83-54.63)
Rash at 18 mo (n = 6313)				
$\chi^2 = 65.44$ ($P = 6.16 \times 10^{-15}$)	27.6%	151/5765	224/542	5/6
OR (95% CI)		1.00	1.98 (1.65-2.37)	4.03 (1.64-120.15)
Rash at 30 mo (n = 6007)				
$\chi^2 = 91.99$ ($P = 1.06 \times 10^{-20}$)	26.8%	1383/5484	222/517	6/6
OR (95% CI)		1.00	2.23 (1.86-2.68)	∞ (4.63-∞)
Rash at 42 mo (n = 5904)				
$\chi^2 = 73.25$ ($P = 1.24 \times 10^{-16}$)	22.8%	1158/5391	186/507	5/6
OR (95% CI)		1.00	2.12 (1.75-2.57)	18.28 (2.13-156.60)
Rash at 57 mo (n = 5663)				
$\chi^2 = 54.54$ ($P = 1.44 \times 10^{-12}$)	24.0%	1182/5173	172/485	5/5
OR (95% CI)		1.00	1.86 (1.52-2.26)	∞ (4.39-∞)
Rash at 81 mo (n = 5361)				
$\chi^2 = 48.15$ ($P = 3.50 \times 10^{-11}$)	19.6%	913/4897	136/460	4/4
OR (95% CI)		1.00	1.83 (1.48-2.27)	∞ (4.54-∞)
Flexural dermatitis at 7 y (n = 5404)				
$\chi^2 = 65.47$ ($P = 6.07 \times 10^{-15}$)	7.3%	337/4925	55/473	5/6
OR (95% CI)		1.00	1.79 (1.32-2.42)	68.07 (7.93-584.31)
Flexural dermatitis at 9 y (n = 4999)				
$\chi^2 = 49.80$ ($P = 1.53 \times 10^{-11}$)	7.6%	312/4593	63/430	3/6
OR (95% CI)		1.00	2.34 (1.75-3.13)	13.63 (2.74-68.79)
Flexural dermatitis at 11 y (n = 4599)				
$\chi^2 = 65.04$ ($P = 7.54 \times 10^{-15}$)	5.7%	211/4204	49/390	3/5
OR (95% CI)		1.00	2.72 (1.96-3.78)	28.39 (4.72-170.80)

AA, Wild type for both mutations; *Aa*, heterozygote for either mutation; *aa*, either a homozygote for each individual mutation (ie, R501X/R501X or 2282del4/2282del4) or a compound heterozygote for each mutation (ie, 2282del4/R501X).

n = Number of cohort for whom all phenotypic and genotypic data are available for each specific phenotypic outcome.

TABLE E4. Interaction between *FLG* alleles and various environmental factors with eczema

	AA	Aa	aa
Boys (n = 2753)			
$\chi^2 = 52.90$ ($P = 3.26 \times 10^{-12}$)	666/2514	113/237	2/2
OR (95% CI)	1.00	2.53 (1.93-3.31)	∞ (2.72-∞)
Girls (n = 2502)			
$\chi^2 = 48.49$ ($P = 2.96 \times 10^{-11}$)	565/2282	95/216	4/4
OR (95% CI)	1.00	2.39 (1.79-3.17)	∞ (2.50-∞)
P for sex interaction = .959			
Mother smoked during pregnancy (n = 717)			
$\chi^2 = 17.59$ ($P = 2.74 \times 10^{-5}$)	157/660	28/57	0/0
OR (95% CI)	1.00	3.09 (1.79-3.36)	
Mother did not smoke during pregnancy (n = 4423)			
$\chi^2 = 81.13$ ($P = 2.41 \times 10^{-18}$)	1047/4028	176/389	6/6
OR (95% CI)	1.00	2.35 (1.90-2.91)	∞ (5.37-∞)
P for smoking interaction = .362			
Child ETS exposure (n = 1035)			
$\chi^2 = 24.53$ ($P = 4.70 \times 10^{-6}$)	234/951	40/83	1/1
OR (95% CI)	1.00	2.85 (1.81-1.49)	∞ (1.96-∞)
No child ETS exposure (n = 3839)			
$\chi^2 = 68.7$ ($P = 1.21 \times 10^{-15}$)	915/3496	153/338	5/5
OR (95% CI)	1.00	2.33 (1.86-2.93)	∞ (3.17-∞)
P for ETS interaction = .742			
Maternal asthma (n = 576)			
$\chi^2 = 26.52$ ($P = 1.74 \times 10^{-6}$)	151/513	25/60	0/3
OR (95% CI)	1.00	3.36 (1.94-5.80)	0 (0-33.56)
No maternal asthma (n = 4612)			
$\chi^2 = 70.65$ ($P = 4.55 \times 10^{-16}$)	1067/4219	171/390	3/3
OR (95% CI)	1.00	2.31 (1.87-2.85)	∞ (3.21-∞)
P for asthma interaction = .486			
Maternal eczema (n = 1228)			
$\chi^2 = 24.97$ ($P = 3.73 \times 10^{-6}$)	363/1082	75/143	3/3
OR (95% CI)	1.00	2.19 (1.54-3.11)	∞ (2.96-∞)
No maternal eczema (n = 3960)			
$\chi^2 = 65.02$ ($P = 7.67 \times 10^{-15}$)	855/3650	131/307	3/3
OR (95% CI)	1.00	2.43 (1.92-3.09)	∞ (2.67-∞)
P for eczema interaction = .884			
Parity of 0 (n = 2401)			
$\chi^2 = 34.06$ ($P = 4.03 \times 10^{-8}$)	570/2215	81/184	2/2
OR (95% CI)	1.00	2.27 (1.67-3.08)	∞ (2.86-∞)
Parity of ≥1 (n = 2062)			
$\chi^2 = 67.13$ ($P = 2.65 \times 10^{-15}$)	661/2581	127/269	4/4
OR (95% CI)	1.00	2.60 (2.01-3.35)	∞ (2.31-∞)
P for parity interaction = .802			
Child breast-fed (n = 4124)			
$\chi^2 = 79.36$ ($P = 5.85 \times 10^{-18}$)	993/3772	161/346	6/6
OR (95% CI)	1.00	2.44 (1.95-3.05)	∞ (2.58-∞)
Child never breast-fed (n = 1034)			
$\chi^2 = 20.35$ ($P = 6.45 \times 10^{-6}$)	207/938	41/96	0/0
OR (95% CI)	1.00	2.63 (1.71-4.06)	
P for breast-feeding interaction = .952			

AA, Wild type for both mutations; *Aa*, heterozygote for either mutation; *aa*, either a homozygote for each individual mutation (ie, R501X/R501X or 2282del4/2282del4) or a compound heterozygote for each mutation (ie, 2282del4/R501X); *ETS*, environmental tobacco smoke.
n = Number of cohort for whom all phenotypic and genotypic data are available for each specific phenotypic outcome.

TABLE E5. Interaction between *FLG* alleles and various environmental factors with asthma

	AA	Aa	aa
Boys (n = 2745)			
χ^2 = 15.05 (P = .001)	246/2518	34/226	1/1
OR (95% CI)	1.00	1.64 (1.11-2.41)	∞ (1.21-∞)
Girls (n = 2486)			
χ^2 = 10.75 (P = .005)	141/2276	25/207	0/3
OR (95% CI)	1.00	2.08 (1.32-3.27)	0 (0-26.17)
P for sex interaction = .731			
Mother smoked during pregnancy (n = 709)			
χ^2 = 2.70 (P = .101)	59/659	8/50	0/0
OR (95% CI)	1.00	1.94 (0.87-4.32)	
Mother did not smoke during pregnancy (n = 4407)			
χ^2 = 14.34 (P = .001)	321/4029	50/374	1/4
OR (95% CI)	1.00	1.78 (1.30-2.45)	3.85 (0.40-37.12)
P for smoking interaction = .850			
Child ETS exposure (n = 984)			
χ^2 = 3.09 (P = .213)	70/917	9/66	0/1
OR (95% CI)	1.00	1.91 (0.91-4.02)	0 (0-33.23)
No child ETS exposure (n = 3726)			
χ^2 = 19.33 ($P = 6.36 \times 10^{-5}$)	276/3401	48/322	1/3
OR (95% CI)	1.00	1.98 (1.43-2.76)	5.66 (0.51-62.63)
P for ETS interaction = .996			
Maternal asthma (n = 570)			
χ^2 = 12.86 (P = .002)	80/510	18/59	1/1
OR (95% CI)	1.00	2.36 (1.29-4.31)	∞ (1.99-∞)
No maternal asthma (n = 4576)			
χ^2 = 7.99 (P = .018)	303/4207	41/366	0/3
OR (95% CI)	1.00	1.63 (1.15-2.30)	0 (0-31.66)
P for asthma interaction = .576			
Maternal eczema (n = 1199)			
χ^2 = 11.64 (P = .003)	110/1065	25/132	2/2
OR (95% CI)	1.00	2.03 (1.26-3.27)	∞ (2.02-∞)
No maternal eczema (n = 3947)			
χ^2 = 6.61 (P = .087)	273/3652	34/293	0/2
OR (95% CI)	1.00	1.63 (1.11-2.37)	0 (0-29.32)
P for eczema interaction = .775			
Parity of 0 (n = 2413)			
χ^2 = 9.38 (P = .009)	160/2225	25/187	0/1
OR (95% CI)	1.00	1.99 (1.27-3.13)	0 (0-33.67)
Parity of ≥1 (n = 2818)			
χ^2 = 8.67 (P = .013)	227/2569	34/246	1/3
OR (95% CI)	1.00	1.66 (1.12-2.44)	5.16 (0.47-57.11)
P for parity interaction = .829			
Child breast-fed (n = 3988)			
χ^2 = 17.48 (P = .0001)	289/3656	47/328	1/4
OR (95% CI)	1.00	1.95 (1.40-2.71)	3.88 (0.40-37.45)
Child never breast-fed (n = 975)			
χ^2 = 1.36 (P = .244)	74/892	10/83	0/0
OR (95% CI)	1.00	1.51 (0.75-3.06)	
P for breast-feeding interaction = .413			

AA, Wild type for both mutations, *Aa*, heterozygote for either mutation; *aa*, either a homozygote for each individual mutation (ie, R501X/R501X or 2282del4/2282del4) or a compound heterozygote for each mutation (ie, 2282del4/R501X); *ETS*, environmental tobacco smoke.
n = Number of cohort for whom all phenotypic and genotypic data are available for each specific phenotypic outcome.

TABLE E6. Interaction between *FLG* alleles and various environmental factors with eczema plus asthma

	AA	Aa	aa
Boys (n = 2359)			
$\chi^2 = 39.64$ ($P = 2.47 \times 10^{-9}$)	94/2162	23/196	1/1
OR (95% CI)	1.00	2.93 (1.81-4.73)	∞ (3.65-∞)
Girls (n = 2135)			
$\chi^2 = 31.84$ ($P = 1.22 \times 10^{-7}$)	53/1954	19/178	0/3
OR (95% CI)	1.00	4.29 (2.48-7.42)	0 (0-30.33)
P for sex interaction = .591			
Mother smoked during pregnancy (n = 571)			
$\chi^2 = 9.09$ ($P = .003$)	17/532	5/39	0/0
OR (95% CI)	1.00	4.46 (1.55-12.81)	
Mother did not smoke during pregnancy (n = 3831)			
$\chi^2 = 45.61$ ($P = 1.24 \times 10^{-10}$)	128/3497	37/330	146
OR (95% CI)	1.00	3.32 (2.26-4.88)	8.77 (1.55-12.81)
P for smoking interaction = .604			
Child ETS exposure (n = 851)			
$\chi^2 = 12.99$ ($P = .002$)	23/791	7/59	0/1
OR (95% CI)	1.00	4.50 (1.84-10.96)	0 (0-31.37)
No child ETS exposure (n = 3408)			
$\chi^2 = 43.66$ ($P = 3.30 \times 10^{-10}$)	118/3111	34/294	1/3
OR (95% CI)	1.00	3.32 (2.22-4.96)	12.68 (1.14-140.85)
P for ETS interaction = .831			
Maternal asthma (n = 481)			
$\chi^2 = 41.07$ ($P = 1.21 \times 10^{-9}$)	30/430	16/50	1/1
OR (95% CI)	1.00	6.28 (3.11-12.64)	∞ (2.97-∞)
No maternal asthma (n = 3970)			
$\chi^2 = 20.44$ ($P = 3.65 \times 10^{-5}$)	117/3646	26/321	0/3
OR (95% CI)	1.00	2.68 (1.71-4.13)	0 (0-34.78)
P for asthma interaction = .388			
Maternal eczema (n = 1063)			
$\chi^2 = 26.86$ ($P = 1.47 \times 10^{-6}$)	51/940	20/121	1/2
OR (95% CI)	1.00	3.45 (1.98-6.02)	17.43 (1.08-282.7)
No maternal eczema (n = 3388)			
$\chi^2 = 22.76$ ($P = 1.14 \times 10^{-5}$)	96/3136	22/250	0/2
OR (95% CI)	1.00	3.06 (1.89-4.95)	0 (0-29.36)
P for eczema interaction = .511			
Parity of 0 (n = 2060)			
$\chi^2 = 35.88$ ($P = 1.62 \times 10^{-8}$)	57/1907	19/152	0/1
OR (95% CI)	1.00	4.64 (2.68-8.02)	0 (0-29.76)
Parity of ≥1 (n = 2432)			
$\chi^2 = 23.3844$ ($P = 3.38 \times 10^{-6}$)	90/2209	23/222	1/34
OR (95% CI)	1.00	2.72 (1.68-4.40)	11.77 (1.06-131.03)
P for parity interaction = .358			
Child breast-fed (n = 3576)			
$\chi^2 = 39.66$ ($P = 2.44 \times 10^{-8}$)	124/3279	33/293	1/4
OR (95% CI)	1.00	3.23 (2.16-4.84)	8.48 (0.89-82.12)
Child never breast-fed (n = 838)			
$\chi^2 = 10.20$ ($P = .00$)	21/767	7/71	0/0
OR (95% CI)	1.00	3.89 (1.59-9.49)	
P for breast-feeding interaction = .168			

AA, Wild type for both mutations; *Aa*, heterozygote for either mutation; *aa*, either a homozygote for each individual mutation (ie, R501X/R501X or 2282del4/2282del4) or a compound heterozygote for each mutation (ie, 2282del4/R501X); *ETS*, environmental tobacco smoke.
n = Number of cohort for whom all phenotypic and genotypic data are available for each specific phenotypic outcome.

TABLE E7. Interaction between *FLG* alleles and various environmental factors with reaction to any of 3 skin prick tests

	AA	Aa	aa
Boys (n = 2470)			
$\chi^2 = 31.70$ ($P = 1.31 \times 10^{-7}$)	242/2270	40/198	2/2
OR (95% CI)	1.00	2.12 (1.46-3.08)	∞ (3.17-∞)
Girls (n = 2294)			
$\chi^2 = 10.41$ ($P = .005$)	159/2090	18/200	2/4
OR (95% CI)	1.00	1.20 (0.72-2.00)	12.15 (1.70-86.79)
P for sex interaction = .210			
Mother smoked during pregnancy (n = 656)			
$\chi^2 = 4.32$ ($P = .038$)	46/606	8/50	0/0
OR (95% CI)	1.00	2.32 (1.03-5.23)	
Mother did not smoke during pregnancy (n = 4006)			
$\chi^2 = 28.76$ ($P = 5.68 \times 10^{-7}$)	347/3658	48/342	4/6
OR (95% CI)	1.00	1.56 (1.13-2.16)	19.08 (3.48-104.6)
P for smoking interaction = .373			
Child ETS exposure (n = 857)			
$\chi^2 = 15.31$ ($P = .00047$)	291/2967	37/268	3/5
OR (95% CI)	1.00	1.47 (1.02-2.13)	13.79 (2.30-82.89)
No child ETS exposure (n = 3240)			
$\chi^2 = 17.82$ ($P = .00011$)	77/786	14/70	1/1
OR (95% CI)	1.00	2.30 (1.23-4.33)	∞ (3.34-∞)
P for ETS interaction = .488			
Maternal asthma (n = 537)			
$\chi^2 = 22.93$ ($P = 1.44 \times 10^{-5}$)	66/480	3/54	3/3
OR (95% CI)	1.00	0.37 (0.11-1.22)	∞ (2.99-∞)
No maternal asthma (n = 4138)			
$\chi^2 = 21.58$ ($P = 2.06 \times 10^{-5}$)	330/3797	54/338	1/3
OR (95% CI)	1.00	2.00 (1.46-2.73)	5.25 (0.48-58.09)
P for asthma interaction = .056			
Maternal eczema (n = 1106)			
$\chi^2 = 21.25$ ($P = 2.43 \times 10^{-5}$)	119/981	17/122	3/3
OR (95% CI)	1.00	1.17 (0.68-2.03)	∞ (2.86-∞)
No maternal eczema (n = 3569)			
$\chi^2 = 14.84$ ($P = .001$)	277/3296	40/270	1/3
OR (95% CI)	1.00	1.90 (1.33-2.71)	5.45 (0.49-60.29)
P for eczema interaction = .355			
Parity of 0 (n = 2135)			
$\chi^2 = 18.09$ ($P = .00012$)	198/1966	33/167	1/2
OR (95% CI)	1.00	2.20 (1.46-3.31)	8.93 (0.56-143.3)
Parity of $\geq$1 (n = 2629)			
$\chi^2 = 23.36$ ($P = 8.47 \times 10^{-6}$)	203/2394	25/231	3/4
OR (95% CI)	1.00	1.31 (0.84-2.03)	32.38 (3.35-312.70)
P for parity interaction = .162			
Child breast-fed (n = 3628)			
$\chi^2 = 27.92$ ($P = 8.65 \times 10^{-7}$)	311/3331	40/291	4/6
OR (95% CI)	1.00	1.55 (1.09-2.20)	19.42 (3.54-106.46)
Child never breast-fed (n = 829)			
$\chi^2 = 3.82$ ($P = .051$)	69/748	13/81	0/0
OR (95% CI)	1.00	1.88 (0.99-3.58)	
P for breast-feeding interaction = .002			

AA, Wild type for both mutations; *Aa*, heterozygote for either mutation; *aa*, either a homozygote for each individual mutation (ie, R501X/R501X or 2282del4/2282del4) or a compound heterozygote for each mutation (ie, 2282del4/R501X); *ETS*, environmental tobacco smoke.
n = Number of cohort for whom all phenotypic and genotypic data are available for each specific phenotypic outcome.

TABLE E8. Associations between *FLG* mutations and potential confounding factors

	R501x		Del4		
	AA (95.8%)	**Aa (4.2%)**	**AA (95.4%)**	**Aa (4.4%)**	**aa (0.1%)**
Sex					
Boy (51.5%)	3454 (95.5%)	164 (4.5%)	3462 (95.6%)	157 (4.3%)	1 (0.01%)
Girl (48.5%)	3223 (96.1%)	130 (3.9%)	3191 (95.2%)	153 (4.6%)	9 (0.03%)
χ^2 (*P* value)	1.85 (.173)		7.28 (.026)		
Mother smoked during pregnancy					
No (80.9%)	5443 (95.8%)	240 (4.2%)	5411 (95.2%)	264 (4.6%)	10 (0.2%)
Yes (19.1%)	1086 (96.2%)	43 (3.8%)	1084 (96.0%)	45 (4.0%)	0
χ^2 (*P* value)	0.41 (.524)		2.95 (.228)		
Child's ETS exposure					
None (74.8%)	3999 (95.7%)	179 (4.3%)	3983 (95.3%)	189 (4.5%)	7 (0.2%)
Prenatal only (0.7%)	40 (100.0%)	0	35 (87.5%)	5 (12.5%)	0
Postnatal only (9.3%)	434 (96.0%)	18 (4.0%)	430 (95.1%)	19 (4.2%)	3 (0.7%)
Both (15.1%)	715 (96.4%)	27 (3.6%)	713 (96.1%)	29 (3.9%)	0
χ^2 (*P* value)	2.45 (.485)		13.82 (.032)		
Maternal asthma					
No (88.7%)	5728 (95.8%)	252 (4.2%)	5717 (95.6%)	255 (4.3%)	9 (0.2%)
Yes (11.3%)	743 (95.9%)	32 (4.1%)	726 (93.7%)	48 (6.2%)	1 (0.1%)
χ^2 (*P* value)	0.01 (.912)		5.98 (.050)		
Maternal hay fever					
No (69.2%)	4485 (95.7%)	29 (4.3%)	4469 (95.4%)	207 (4.4%)	10 (0.2%) 0
Yes (30.8%)	1986 (96.0%)	83 (4.0%)	1974 (95.4%)	96 (4.4%)	
χ^2 (*P* value)	0.28 (.600)		4.57 (.102)		
Maternal eczema					
No (77.2%)	5025 (96.3%)	191 (3.7%)	4991 (95.7%)	219 (4.2%)	7 (0.1%)
Yes (22.8%)	1446 (94.0%)	93 (6.0%)	1452 (94.3%)	84 (5.5%)	3 (0.2%)
χ^2 (*P* value)	16.73 (<.00001)		4.72 (.094)		
Maternal social class*					
I (5.8%)	376 (97.2%)	11 (2.8%)	370 (95.6%)	17 (4.4%)	0
II (31.5%)	1865 (96.3%)	71 (3.7%)	1834 (94.7%)	99 (5.1%)	4 (0.2%)
III NM (43.3%)	2433 (95.7%)	108 (4.3%)	2419 (95.2%)	117 (4.6%)	5 (0.2%)
III M (7.6%)	400 (95.2%)	20 (4.8%)	403 (96.0%)	17 (4.0%)	0
IV/V (11.8%)	583 (94.8%)	32 (5.2%)	593 (96.3%)	23 (3.7%)	0
χ^2 (*P* value)	4.99 (.288)		5.42 (.712)		
Parity					
0 (44.3%)	2863 (96.2%)	113 (3.8%)	2851 (95.8%)	120 (4.0%)	6 (0.2%)
1 (36.2%)	2369 (95.5%)	112 (4.5%)	2365 (95.3%)	115 (4.6%)	2 (0.1%)
≥2 (19.5%)	1250 (95.7%)	56 (4.3%)	1236 (94.6%)	68 (5.2%)	2 (0.2%)
χ^2 (*P* value)	1.82 (.403)		4.48 (.345)		
Child breast-fed					
No (24.%)	1313 (95.9%)	56 (4.1%)	1301 (95.0%)	66 (4.8%)	3 (0.2%)
Yes (75.8%)	4744 (95.9%)	204 (4.1%)	4728 (95.6%)	214 (4.3%)	6 (0.1%)
χ^2 (*P* value)	0.01 (.958)		1.35 (.510)		
Central or storage heating					
No (13.1%)	761 (95.4%)	37 (4.6%)	752 (94.1%)	47 (5.9%)	0
Yes (86.9%)	5768 (95.9%)	249 (4.1%)	5748 (95.5%)	260 (4.3%)	10 (0.2%)
χ^2 (*P* value)	0.44 (.509)		5.29 (.071)		
Maternal age (y)					
<20 (3.4%)	147 (91.9%)	13 (8.1%)	157 (98.1%)	3 (1.9%)	0
20-24 (17.1%)	1000 (95.5%)	47 (4.5%)	1002 (95.7%)	44 (4.2%)	1 (0.1%)
25-29 (39.7%)	2641 (96.0%)	109 (4.0%)	2637 (95.9%)	109 (4.0%)	5 (0.2%)
≥30 (39.9%)	2889 (95.9%)	125 (4.1%)	2857 (94.8%)	154 (5.1%)	4 (0.1%)
χ^2 (*P* value)	6.71 (.082)		7.96 (.241)		

ETS, Environmental tobacco smoke.

*For definitions of social class coding, see Office of Population Censuses and Surveys Standard Occupational Classification. Volumes 1 and 3. London: HMSO; 1990.

TABLE E9. Number of individuals under observation throughout the follow-up period

***FLG* genotype**	**Month 0**	**Month 40**	**Month 80**	**Month 120**
Wild type	1901	1422	530	123
Aa or aa	296	250	122	41

AA, Wild type for both mutations, *Aa*, heterozygote for either mutation; *aa*, either a homozygote for each individual mutation (ie, R501X/R501X or 2282del4/2282del4) or a compound heterozygote for each mutation (ie, 2282del4/R501X).