Filaggrin null mutations and childhood atopic eczema: A population-based case-control study

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Background: Null mutations within the filaggrin gene *(FLG)* are associated with moderate-to-severe atopic eczema; their role in mild-to-moderate eczema in the general population is unknown.
Objective: We sought to investigate the significance of 5 common *FLG* null mutations in childhood atopic eczema in an unselected population cohort.
Methods: Eight hundred eleven English children aged 7 to 9 years were screened for *FLG* mutations. Eczema cases were defined by using United Kingdom diagnostic criteria and skin examination. Asthma and seasonal rhinitis cases were defined by parental questionnaire. Association between phenotype and genotype was investigated using Fisher exact test and logistic regression analysis.
Results: The 12-month period prevalence of atopic eczema was 24.2% (95% CI, 21.2% to 27.2%), with 96% (115/120) of cases having mild-to-moderate disease. The combined null genotype (carriage of ≥1 *FLG* mutations) was significantly associated with atopic eczema ($P = 1.2 \times 10^{-4}$). The odds ratio (OR) for individuals carrying 2 null mutations was 26.9 (95% CI, 3.3-217.1), but heterozygote carriers showed no significant increase in risk (OR, 1.2; 95% CI, 0.7-1.9). Eight of 190 eczema cases (4.2%) carried 2 *FLG* null mutations and thus might be attributed to filaggrin deficiency. Asthma in the context of eczema showed significant association with the *FLG* null mutations ($P = 7.1 \times 10^{-4}$). There was no association of *FLG* with asthma independent of eczema ($P = .15$) and no association with seasonal rhinitis ($P = .66$).
Conclusion: *FLG* null mutations are significantly associated with mild-to-moderate atopic eczema in childhood, with a recessive pattern of inheritance. (J Allergy Clin Immunol 2008;121:940-6.)

Key words: *Asthma, seasonal rhinitis, atopic eczema, complex trait, filaggrin, ichthyosis vulgaris, skin barrier function*

Atopic eczema[1] is an itchy inflammatory skin condition that follows a chronic relapsing course. It can cause significant morbidity as a result of pruritus, sleep deprivation, and emotional distress.[2]

Eczema is a complex trait; that is, multiple genetic and environmental factors contribute to the phenotype. There is an increasing recognition of the role of epithelial barrier dysfunction in the pathogenesis of atopic eczema,[3,4] and it has been suggested that allergen penetration might predate the development of asthma and allergic rhinitis[3] in the so-called *atopic march*.[5]

Filaggrin (filament-aggregating protein) plays a key role in epidermal differentiation and skin barrier function. Filaggrin aggregates the keratin cytoskeleton to facilitate the collapse and flattening of keratinocytes in the outermost skin layer.[6] The protein-lipid cornified envelope, which replaces the plasma membrane of terminally differentiated keratinocytes, is extensively cross-linked and forms an important barrier to prevent water loss and minimize the entry of allergens and microorganisms.[7] Filaggrin is subsequently degraded to produce a mixture of hygroscopic amino acids, which can also contribute to barrier function.[8] Genotypes resulting in relative or absolute filaggrin deficiency might therefore contribute to epidermal barrier dysfunction by more than 1 mechanism.

At least 15 different loss-of-function (null) mutations in the filaggrin gene *(FLG)* have been reported to date, of which 5 are prevalent in the European population.[9] *FLG* null mutations cause the dry, scaly skin condition ichthyosis vulgaris[10]; the 5 most common European mutations are also significantly associated with atopic eczema when analyzed individually and as a combined null genotype.[9] The association with atopic eczema has been replicated in at least 12 separate studies, most of which contain further within-study replication, with no negative or equivocal results,[11] making this an unusually well-replicated finding in the field of complex genetics.

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Abbreviations used
FLG: Filaggrin gene
OR: Odds ratio
UK: United Kingdom

The clinical phenotype of eczema is heterogeneous and likely to encompass considerable etiologic heterogeneity.[12] The studies published to date looking at *FLG* null mutations have focused on moderate-to-severe atopic eczema cases recruited through specialist clinics[9,13-20] and on children with eczema as part of atopy-related birth cohort studies.[13,15] We therefore aimed to investigate whether the 5 most prevalent *FLG* null mutations make a significant contribution to the mild-to-moderate atopic eczema that is common in the English population. We also aimed to assess the association of *FLG* null mutations with other signs of dry skin (eg, xerosis, ichthyosis, keratosis pilaris, and hyperlinear palms) to further define the filaggrin-deficiency phenotype.

METHODS

Study design and case definition

We used a population cohort (n = 7737) from the northwest of England for whom DNA samples had been collected at birth,[21] representing more than 85% of all deliveries in one hospital (West Cumberland Hospital, Whitehaven) between 1996 and 2003. This geographic area is particularly useful for epidemiologic research because of the relatively low rates of population movement. Children within the birth cohort catchment region who had not previously been included in the cohort were offered the opportunity to provide a saliva sample for DNA extraction so that they could be included in this study.

Children between 7 and 9 years of age (n ≈ 4000) were chosen for this study as an optimal group in which to assess atopic disease (eczema, asthma, and seasonal rhinitis). Eczema is most prevalent in infancy and early childhood; 85% of children are affected before the age of 5 years,[22] and 50% to 70% of children "grow out" of their eczema by the age of 10 years,[23,24] although they have an increased risk of recurrence in adulthood,[25] as well as an increased incidence of asthma and rhinitis.[5] Asthma shows a peak age of onset of 5 years, and therefore the majority of cases should be captured in our study age group.

The study protocol was approved by the local research ethics committee, and a parent or guardian of each child provided written informed consent.

All 57 primary and junior schools within the birth cohort catchment area were requested to participate in the study to allow distribution of questionnaires to all children aged 7 to 9 years. Questionnaire design used the well-validated United Kingdom (UK) Diagnostic Criteria[26] to assess the 12-month period prevalence of eczema (the full questionnaire appears as Fig E1 in the Online Repository at www.jacionline.org). Additional, more detailed phenotypic data were gathered by a single dermatologist (S.J.B.) who had experience of working in a pediatric dermatology clinic. The skin on each child's face, limbs, and abdomen was examined. Eczema cases were defined according to the UK diagnostic criteria (representing a 12-month period prevalence), flexural eczema on examination by the dermatologist (a point prevalence), or both. Children not included in either of these categories were classified as control subjects.

Eczema was graded by using the Three Item Severity score,[27] a simple validated scale that allows the rapid assessment and recording of clinically significant features[28]: 1 to 2 represents mild eczema, 3 to 5 represents moderate eczema, and 6 to 9 represents severe eczema.[29]

The association between *FLG* mutations and the other atopic disorders, asthma and seasonal rhinitis, was investigated as a secondary analysis. Asthma and seasonal rhinitis prevalence were defined by parental replies to the following questions: "Has your child ever suffered from asthma? By asthma we mean bouts of wheezing and coughing" and "Has your child ever suffered from hay fever? By hay fever we mean bouts of sneezing with a runny nose or itchy eyes in the summer."[26]

Genotypic analysis

DNA was extracted from umbilical cord blood or umbilical cord tissue from the birth cohort individuals using standard procedures. Saliva samples were collected from the remainder using Oragene DNA self-collection kits.

DNA samples were screened for the 5 *FLG* null mutations most prevalent in the European population,[9] as previously described: TaqMan allelic discrimination assay (Applied Biosystems, Foster City, Calif) for R501X, 2282del4, R2447X, and S3247X and size analysis of fluorescently labeled PCR products using an Applied Biosystems 3100 DNA sequencer for 3702delG.[30] Homozygote and heterozygote results were confirmed by means of restriction enzyme digestion[10,30] or sequencing.[9]

Statistical analysis

Calculations based on an estimated 11% prevalence of eczema[25] and combined null allele frequency of 0.078[13] predicted that recruitment of 1000 children would give a 91% power at a *P* value of .001 to detect an allelic odds ratio (OR) of 3 (at the lower end of published data[13,15]). A similar calculation predicted that there would be insufficient power to detect an association between each individual mutation and eczema.

The rationale for counting the 5 screened *FLG* null mutations as a single null allele is based on biochemical and immunohistochemical studies demonstrating that each of these null mutations produce truncated forms of profilaggrin, which results in a marked reduction or absence of processed filaggrin when present in the homozygote or compound heterozygote state.[9,10]

Allele and genotype frequencies in case and control groups and different phenotypic groups were compared by using the Fisher exact test under the null hypothesis that there is no association with genotype. Logistic regression analysis was used to estimate the OR and penetrance of the *FLG* null allele using both allele- and genotype-based models of disease to investigate the pattern of inheritance. Analysis was performed with the statistical analysis package Stata (version 9, Stata for Linux; StataCorp LP, College Station, Tex).

RESULTS

Seventy-five percent (43/57) of eligible schools agreed to take part in the research by distributing study literature and facilitating skin examinations by the visiting dermatologist. An estimated 3086 children in these schools were within the target age range, and consent to participate was given by the parents/guardians of 811 (26%) children. Eight hundred one questionnaires were completed, 792 children underwent skin examination, and 805 DNA samples were located from the birth cohort stores (n = 577) or extracted from saliva (n = 228), giving complete phenotypic and genotypic information on 784 individuals (Fig 1). Individuals with incomplete phenotypic data, genotypic data, or both were included in the statistical analyses unless the missing data precluded this (eg, insufficient criteria to define UK diagnosis or insufficient genotyping results to identify the combined genotype).

The demographic data for this cohort plus clinical features relating to eczema and atopy are summarized in Table I.

Screening for all 5 mutations achieved results for 789 of 805 DNA samples (Table II). Approximately 1 in 7 children (14.2%) of the study cohort are carriers of 1 or more of the *FLG* mutations. Of eczema cases, 18.4% carried 1 or more *FLG* null mutations compared with 12.9% of control subjects. The combined null genotype is significantly associated with atopic eczema ($P = 1.2 \times 10^{-4}$).

Genotyping results can be analyzed by using either a genotypic model (comparing frequencies of wild-type with heterozygote and homozygote mutants) or using an allele-based model (which presumes that carriage of 2 mutations results in twice the effect of 1 mutation on a log odds scale). A comparison of these 2 models for our data is shown in Fig 2. The genotypic model fits the data

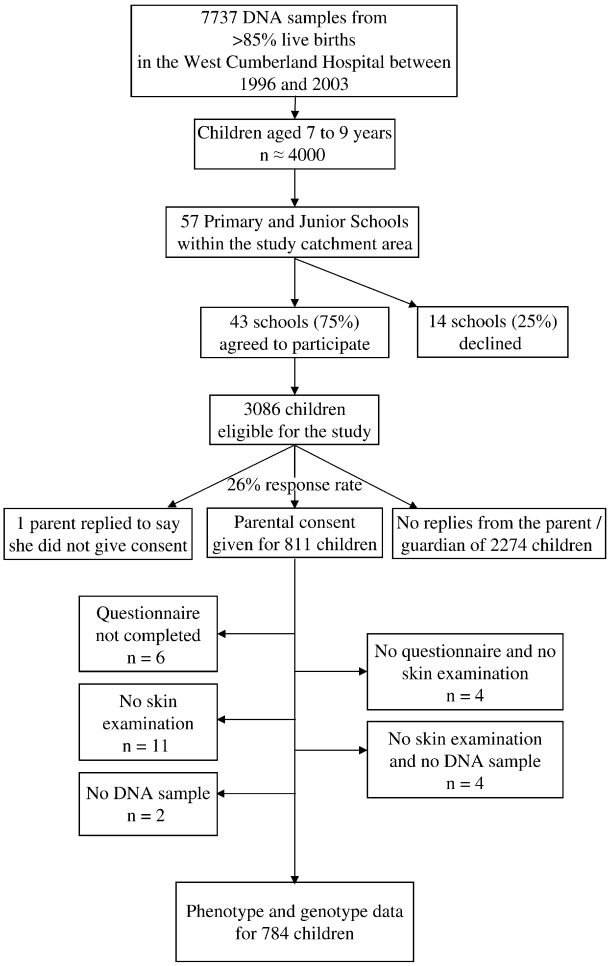

FIG 1. Flow diagram showing recruitment of children to the study.

TABLE I. Demographic data and clinical features of 811 children aged 7 to 9 years

Demographic data	No.	Percentage
Sex	417 male	51
	394 female	49
Prevalence of atopic eczema	195/806	24
Eczema severity	70/120 mild	58
	45/120 moderate	38
	5/120 severe	4
Prevalence of asthma	205/794	26
Children with eczema and asthma	83/793	11% of total cohort
		43% of eczema cases
		41% of asthma cases
Prevalence of seasonal rhinitis	152/793	19

The prevalence of atopic eczema was defined using a questionnaire based on the UK diagnostic criteria[26] (12-month period prevalence) plus skin examination by a dermatologist (point prevalence). Eczema severity was assessed using the Three Item Severity score.[27] The lifetime prevalence of asthma and seasonal rhinitis was defined by parental questionnaire. The denominator varies to maximize the use of available data.

TABLE II. *FLG* genotypes in a cohort of English schoolchildren compared by using the Fisher exact test

		Atopic eczema	
		No. (%) of cases	No. (%) of control subjects
FLG null genotype	AA	155 (81.6)	522 (87.1)
	Aa	27 (14.2)	76 (12.7)
	aa	8 (4.2)	1 (0.2)
	Total	190	599
Fisher exact test		$P = 1.2 \times 10^{-4}$	

A, Wild-type form of *FLG*; *a*, null mutant form, either R501X, 2282del4, R2447X, S3247X, or 3702delG; *aa*, individuals including 1 homozygote (R501X/R501X) and 8 compound heterozygotes (6 are R501X/2282del4, including the aa in the control group; 1 is 2282del4/R2447X; and 1 is R501X/R2447X). Each of the 5 variants is in Hardy-Weinberg equilibrium within the cohort.
Complete genotypic data (results on screening for all 5 mutations) were obtained on a total of 789 children. The genotyping results for each individual mutation are available in Table E1 in this article's Online Repository at www.jacionline.org.

significantly better ($P = .0018$, likelihood ratio test), and hence this model is preferable because it makes fewer assumptions about the mode of inheritance. This indicates that *FLG* null mutations have a recessive mode of inheritance because the heterozygotes have no significant increased risk of eczema (OR, 1.2; 95% CI, 0.7-1.9), but individuals carrying 2 copies of the *FLG* null mutations (homozygotes and compound heterozygotes) have an approximately 27 times greater risk of having childhood eczema (OR, 26.9; 95% CI, 3.3-217.1).

There was no association between parental report of asthma ($P = .15$) or seasonal rhinitis ($P = .66$) and *FLG* null mutations, but there was evidence of an association between the phenotype of asthma with eczema, which was significant even after allowing for multiple testing ($P = 7.1 \times 10^{-4}$; OR for carriers of 2 null mutations, 11.9; 95% CI, 3.1-45.6). Of children with eczema and asthma, 23.2% carry 1 or more of the *FLG* null mutations compared with 11.8% of control subjects. The results of logistic regression analysis and the Fisher exact test to test association of these and other phenotypes with the *FLG* genotype are summarized in Table III.

In our cohort 10 of 792 children had classical ichthyosis vulgaris (fine gray-white scaling on extensor skin surfaces plus hyperlinear palms and keratosis pilaris) on clinical examination, a prevalence of approximately 1 in 80. One was homozygous for the R501X mutation, 6 were compound heterozygotes, and 3 were heterozygotes (each carrying the R501X mutation). Three of the 10 patients with ichthyosis vulgaris had no eczema by either of our definitions and no parental report of eczema at any time in the child's life, and interestingly, these were the 3 R501X heterozygotes. There were a further 54 children with milder ichthyosis (scaling of the skin), as well as 193 with xerosis (dryness of the skin).

Looking at the genotype-phenotype correlation, all 9 of the homozygous/compound heterozygous individuals in our cohort had ichthyosis vulgaris or milder ichthyosis, and 8 of these 9 had eczema. Conversely, in the 103 heterozygous individuals, 75 (73%) did not have ichthyosis vulgaris or milder ichthyosis, but 20 (27%) of this subgroup (heterozygotes without any ichthyosis) did have eczema. However, using logistic regression to model the effect of genotype on eczema status, having controlled for the effect of ichthyosis vulgaris, showed that any additional effect of *FLG* genotype is not statistically significant ($P = .43$).

The physical signs of hyperlinear palms and keratosis pilaris in childhood are each strongly and significantly associated with the

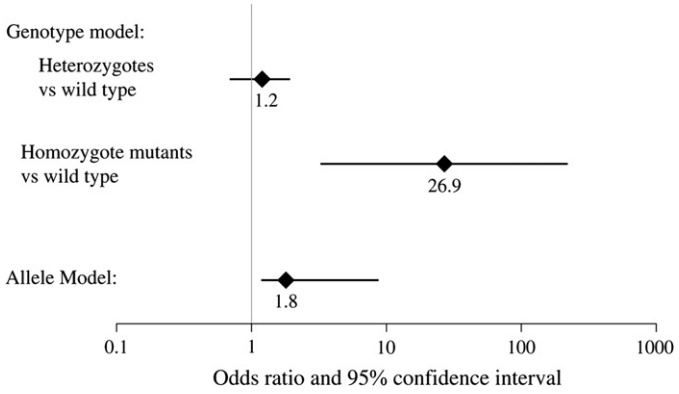

FIG 2. Logistic regression analysis to estimate odds of disease (atopic eczema) with *FLG* null mutations comparing genotype- and allele-based models. The 95% CIs are as follows: genotypic model heterozygotes versus wild type, 0.7 to 1.9; homozygote mutants versus wild type, 3.3 to 217.1; allelic model, 1.2 to 8.6. The likelihood ratio test, to test whether the allelic model can be nested in full within the genotypic model of disease, shows that the genotypic model fits the data significantly better ($P = 1.8 \times 10^{-3}$).

combined *FLG* null genotype (Table III). Marked hyperlinear palms also show significant association with the 4 most prevalent *FLG* null mutations (R501X, 2282del4, R2447X, and S3247X) when they are analyzed individually ($P < .01$ for each variant). Similarly, marked keratosis pilaris shows significant and independent association with the 2 most prevalent null mutations (R501X and 2282del4, $P < .01$ for each). These figures translate into a positive predictive value of 71% for marked hyperlinear palms; that is, 71% of children with marked hyperlinear palms carry 1 or more of the 5 common *FLG* null mutations. The negative predictive value is 90%, meaning 90% of children without hyperlinear palms are not carriers of these mutations. The predictive values for marked keratosis pilaris in this age group are 53% (positive) and 90% (negative predictive value).

DISCUSSION

There have, to date, been no published studies investigating the role of *FLG* mutations in the mild-to-moderate eczema that predominates in our society. We used a unique resource in the northwest of England, an unselected birth cohort with DNA samples, to investigate this question. We were careful to define eczema using a well-validated method (the UK modification of Hanifin and Rajka's diagnostic criteria)[26] in addition to skin examination by an experienced dermatologist. This allowed us to gather more detailed information on phenotypic features, such as xerosis, ichthyosis and keratosis pilaris, which have not been documented in previous studies. Diagnostic accuracy and careful phenotype documentation are particularly important in genetic studies if individual genetic factors are to be identified on the background of complex environmental effects.[31]

The low response rate of only 26% reflects our method of recruitment, requiring informed consent before questionnaire completion.[32] This raises the possibility that bias has been introduced by self-selection in our study cohort. We addressed this question in 2 ways.

First, comparison of the prevalence of eczema and asthma in our cohort with a recently published study of atopy prevalence in the northeast of England by using a case definition based on the International Study of Asthma and Allergies in Childhood questionnaire (90% response rate, n = 3000, 6- to 7-year-olds)[33] showed very similar findings: "rash with typical distribution," 21.1% for boys and 23.8% for girls; "current rash," 23.3% for boys and 25.0% for girls compared with a prevalence of 24.2% in our study (boys and girls combined). Similarly, asthma lifetime prevalence from this comparable study is reported as 29.8% for boys and 24.1% of girls compared with 26.2% in our study.

Second, we compared the allele frequencies for each of the 5 *FLG* mutations in our study cohort with those of an unselected 1000 samples from age-matched children in the same birth cohort (screened as part of another study[34]) and found no significant difference ($P > .05$, Fisher exact test; these data are available in Table E2 in the Online Repository at www.jacionline.org). We therefore conclude that our cohort is representative of the local population, and hence we can use it to estimate the true risk (or penetrance) associated with *FLG* mutations and not just their relative risk, which would be estimated from a selected case-control study. From our data, we calculate that the probability of disease (ie, having mild-to-moderate eczema) is 22.9% in wild-type individuals (95% CI, 19.7% to 26.1%), 26.2% in heterozygotes (95% CI, 17.7% to 34.7%), and 88.9% (95% CI, 68.4% to 109.4%) for homozygotes.

In our cohort the prevalence of ichthyosis vulgaris is approximately 1 in 80. This is significantly higher than that reported in the only previous population cohort study, when 1 in 250 English schoolchildren were found to have the disorder.[35] This higher prevalence might reflect more careful disease ascertainment with a dermatology specialist. Furthermore, the prevalence of *FLG* null mutations in the population (9/789 individuals carrying 2 mutations) would support these examination findings, with a predicted prevalence of ichthyosis vulgaris of approximately 1 in 88 (presuming 100% penetrance and not including the effect of semidominant inheritance). Ichthyosis vulgaris and milder ichthyosis were each significantly associated with *FLG* status in our cohort. This is consistent with data from previous studies that *FLG* shows semidominant inheritance (ie, heterozygote carriers tend to have a milder phenotype than homozygotes).[13]

This study adds to our understanding of the genetic basis of eczema in several ways. The phenotype studied (mild-to-moderate atopic eczema in 7- to 9-year-olds) is significantly associated with *FLG* loss-of-function mutations in the population of the

TABLE III. Prevalence, logistic regression ORs, and *P* values for the Fisher exact test for different phenotypes and the combined *FLG* null genotype

Phenotype or clinical feature	No. (denominator)	OR (95% CI)	Fisher exact test
Atopic eczema (UK criteria + flexural)	195 (806)	Hets: 1.2 (0.7–1.9)	$P = 1.2 \times 10^{-4}$
		Homs: 26.9 (3.3–217.1)	
Nonflexural eczema*	35 (792)	Hets: 0.4 (0.1–1.8)	$P = .52$
		Homs: †	
Asthma	205 (794)	Hets: 1.0 (0.6–1.6)	$P = .15$
		Homs: 3.6 (1.0–13.7)	
Atopic eczema with asthma	83 (793)	Hets: 1.5 (0.8–2.8)	$P = 7.1 \times 10^{-4}$
		Homs: 11.9 (3.1–45.6)	
Seasonal rhinitis	152 (793)	Hets: 1.2 (0.7–2.0)	$P = .66$
		Homs: 0.5 (0.1–4.4)	
Ichthyosis vulgaris	10 (792)	**Hets: >100**‡	$P = 4.1 \times 10^{-16}$
		Homs: >100‡	
Milder ichthyosis	54 (792)	**Hets: 5.5** (3.0–10.0)	$P = 1.7 \times 10^{-7}$
		Homs: 5.8 (1.2–29.1)	
Xerosis	193 (792)	Hets: 1.5 (0.9–2.4)	$P = .04$
		Homs: †	
Hyperlinear palms (marked)	52 (792)	**Hets: 17.5** (9.0–34.2)	$P = 1.2 \times 10^{-23}$
		Homs: 152.1 (29.1-794.3)	
Hyperlinear palms (any)	167 (792)	**Hets: 19.3** (11.7–31.7)	$P = 6.8 \times 10^{-42}$
		Homs: †	
Keratosis pilaris (marked)	77 (792)	**Hets: 8.9** (5.2–15.2)	$P = 2.8 \times 10^{-18}$
		Homs: 35.1 (8.4–146.1)	
Keratosis pilaris (any)	273 (792)	**Hets: 3.6** (2.3–5.5)	$P = 2.2 \times 10^{-12}$
		Homs: †	

Hets, Individuals who are heterozygous for any of the 5 *FLG* null mutations (R501X, 2282del4, R2447X, S3247X, and 3702delG); *Homs*, individuals with any 2 *FLG* mutations, including homozygotes and compound heterozygotes.

Atopic eczema is defined by using the UK diagnostic criteria[26] and skin examination by a dermatologist; asthma and seasonal rhinitis are defined by parental questionnaire. The diagnoses of ichthyosis vulgaris (scaly skin on extensor surfaces plus hyperlinear palms and keratosis pilaris), milder ichthyosis (scaly skin), and xerosis (dry skin) are based on clinical examination by a dermatologist on a single occasion for each child during the winter months. These analyses use the genotypic model of disease, which is less powerful than the allele-based model but makes no assumption about the mode of inheritance, which is currently unknown. Statistically significant results ($P < .01$) are highlighted in boldface.

*Cases with nonflexural eczema only (ie, no flexural involvement).

†OR cannot be estimated because there are no homozygotes in the control group.

‡Insufficient data for reliable estimation of OR.

northwest of England. This contrasts with the cases of nonflexural eczema within the cohort, a group likely to include those with discoid eczema and contact dermatitis, who do not show an association with *FLG* null mutations, although the numbers are too small to draw firm conclusions.

In these cases of predominantly mild-to-moderate atopic eczema, *FLG* shows a recessive pattern of inheritance, rather than the semidominant pattern reported in the original group of eczema cases in families with ichthyosis vulgaris.[13] The fact that we only have 9 individuals in our cohort that are homozygous for *FLG* null mutations is reflected in the wide confidence intervals around the ORs in Fig 2. However, we can still be confident that the data demonstrate a recessive pattern of inheritance because 8 (4.0%) of the 190 cases carry 2 *FLG* null mutations compared with only 1 (0.2%) of 599 control subjects, and hence these 9 individuals provide good evidence for a strong homozygote effect. The CIs do not overlap, and there is a very small chance ($P = 1.8 \times 10^{-3}$) of observing these data under an allele-based model (semidominant inheritance) rather than a genotype-based model (recessive inheritance).

The association of mild-to-moderate eczema with *FLG* is closely related to the presence of ichthyosis vulgaris because logistic regression modeling showed no additional effect of genotype, having allowed for the presence/absence of ichthyosis vulgaris. This is perhaps unsurprising, given that *FLG* mutations are known to cause ichthyosis vulgaris and that 50% to 70% of patients with ichthyosis vulgaris also have eczema. This chain of reasoning emphasizes the role of epidermal barrier dysfunction, which is characteristic of filaggrin deficiency, in the pathogenesis of eczema. An alternative explanation is that *FLG* is associated with eczema only through linkage disequilibrium with another gene. However, this seems unlikely given the independent association of 5 different *FLG* mutations with atopic eczema in the European population[9,30] and Asian-specific *FLG* null mutations that have arisen independently from the European variants,[36] as well as a reduction in filaggrin expression in atopic skin[37] and biochemical studies demonstrating a similar reduction/absence of processed filaggrin as a result of *FLG* null mutations.[10]

The OR of having moderate-to-severe eczema given the presence of 1 or more *FLG* mutations has varied between 2.03 and 13.4 in previous reports.[11,13,20] The equivalent OR for our predominantly mild-to-moderate cases would be 1.53 (95% CI, 0.99-2.37) if we had used a dominant model of analysis (combining the heterozygotes with the homozygote mutants to compare carriers of ≥ 1 *FLG* null mutations with wild-type individuals). This lower OR indicates a lesser effect of the *FLG* mutations in

mild-to-moderate eczema compared with moderate-to-severe eczema. Possible explanations for this observation include an intuitive "dilution" of effect in the less severe phenotype or a greater effect of other factors, genetic and environmental, which are relatively more important than filaggrin insufficiency in milder eczema. The dominant model gives a nonsignificant result for our data (95% CI overlapping 1.0) because of the large number of heterozygotes and small number of homozygotes in the cohort.

Recognition of the relative importance of different causative factors in the mild-to-moderate compared with the moderate-to-severe eczema phenotype might be helpful in designing more rational therapies aimed at targeting specific pathology.

Interestingly, the frequency of carriage of *FLG* mutations in our cases (18.4%) is similar to that reported in children from a Danish birth cohort whose mothers had asthma (n = 372) in which 17.5% of eczema cases were carriers of 1 or more *FLG* null alleles.[13] In these children there was a strikingly high penetrance: 63% of carriers had atopic eczema by the age of 3 years, suggesting that other influential factors (genetic, environmental, or both) are associated with the maternal history of asthma. Our cohort shows an association of *FLG* mutations with asthma only in those children who also have eczema, replicating earlier reports[13,15] and emphasizing the existence of a subgroup of asthma in association with eczema.

This study demonstrates the usefulness of careful physical examination of the skin because hyperlinear palms and keratosis pilaris are features that can help to predict the presence of an *FLG* null mutation. The mechanisms by which filaggrin insufficiency produces these clinical signs remain to be elucidated.

Finally, in spite of the significant effect demonstrated in this cohort, *FLG* null mutations can only explain a small proportion of the total burden of childhood eczema on a population scale. Eight (4.2%) of 190 atopic eczema cases in our cohort carry 2 null mutations and thus can be attributed to *FLG* deficiency. The residual 95.8% of cases remain to be explained by other genetic and environmental factors. However, *FLG* is the single most significant genetic factor in atopic eczema that has been identified to date, and our study supports a significant role for filaggrin insufficiency in the pathogenesis of a small proportion of common, mild-to-moderate atopic eczema cases.

We thank the schools in West Cumbria, the children, and their parents for participating in this research.

Clinical implications: This finding emphasizes the role of ichthyosis vulgaris and filaggrin deficiency in the pathogenesis of approximately 4% of eczema cases in the English population.

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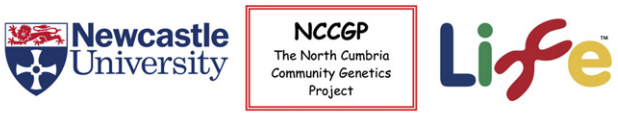

ECZEMA IN CHILDREN
Parent/Guardian Questionnaire

If you would like your child to take part in this research study, we also need to ask the following few questions.
It should not take you more than 5 or 10 minutes to complete this questionnaire.

- Child's full name…… • Child's date of birth……..…..
- Child's school…… • Class……..……
- Mother's name…… • Mother's date of birth……..…..
  (so that we can check if there is a sample for your child with the NCCGP)
- The address where the child's mother was living when the child was born……..……
  ……..……
- Child's current address……..……..
  ……
- Has your child had an **itchy skin** condition in the past 12 months? ☐**YES** ☐**NO**
  If **YES** → How old was your child when this skin condition began? *(Please tick one box)*
  ☐**Under 2 years** ☐**2 to 5 years** ☐**6 years or over** ☐**I can't remember**
  And has this skin condition EVER affected the skin creases? ☐**YES** ☐**NO**
  By skin creases we mean fronts of elbows, behind the knees, fronts of ankles, around the neck or around the eyes.
- Has your child ever at any time suffered from **eczema**? ☐**YES** ☐**NO**
  By eczema we mean patches of red, itchy skin which may be dry and scaly or wet and weeping.
- Has your child ever suffered from **asthma**? ☐**YES** ☐**NO**
  By asthma we mean bouts of wheezing with coughing.
- Has your child ever suffered from **hay fever**? ☐**YES** ☐**NO**
  By hay fever we mean bouts of sneezing with a runny nose or itchy eyes in the summer.
- Does anyone in your child's immediate family suffer from **eczema, asthma** or **hayfever**? ☐**YES** ☐**NO**
  By immediate family we mean the child's mother, father, brother or sister.
- Does your child suffer from **generally dry skin**? ☐**YES** ☐**NO**
- Has your child had any other skin problems? *You can write them here*……
  ……

FIG E1. Questionnaire used in this study.

TABLE E1. Genotyping results for all 5 *FLG* null mutations and the combined null genotype in a cohort of 805 English schoolchildren

	R501X		2282del4		R2447X		S3247X		3702delG		Combined null genotype	
Genotype	**Control population**	**Atopic eczema cases**	**Control population**	**Atopic eczema cases**	**Control population**	**Atopic eczema cases**	**Control population**	**Atopic eczema cases**	**Control population**	**Atopic eczema cases**	**Control population**	**Atopic eczema cases**
AA	566	177	572	176	592	189	598	192	599	191	522	155
Aa	36	16	29	18	8	3	4	3	0	1	76	27
Aa	0	1	0	0	0	0	0	0	0	0	1	8
Total	602	194	601	194	601	192	602	195	599	192	599	190
Fisher exact test	$P = .11$		$P = .03$		$P = .73$		$P = .37$		$P = .24$		$P = 1.2 \times 10^{-4}$	
MAF cases vs control subjects	0.046	0.030	0.046	0.024	0.008	0.006	0.008	0.003	0.003	0.000	0.110	0.065
MAF combined cases and control subjects	0.034		0.030		0.007		0.004		0.001		0.077	
OR using the genotypic model (95% CI)												
Hets	1.4 (0.8-2.6)		2.0 (1.1-3.7)		1.2 (0.3-4.5)		2.3 (0.5-10.5)		NA		1.2 (0.7-1.9)	
Homs	NA		NA		NA		NA		NA		26.9 (3.3-217.1)	
OR using the allelic model (95% CI)	1.6 (0.9-2.8)		2.0 (1.1-3.7)		1.2 (0.3-4.5)		2.3 (0.5-10.5)		NA		1.8 (1.2-2.6)	
LR test	—		—		—		—		—		$P = .0018$	

AA, Homozygous wild-type genotype; *Aa*, heterozygous wild-type/mutant genotype for any of the 5 *FLG* null mutations (R501X, 2282del4, R2447X, S3247X, or 3702delG); *aa*, homozygous or compound heterozygous mutant for any of the 5 *FLG* null mutations; *MAF*, minor allele frequency; *NA*, not analyzed because of absent or insufficient numbers of homozygotes; *LR*, likelihood ratio test to compare genotype and allelic models.

TABLE E2. *FLG* minor allele frequencies in a cohort of English schoolchildren and an unselected age-matched population birth cohort from the same geographic area

	Minor allele frequency (95% CIs)	
***FLG* mutation**	**Schoolchildren participating in the study (n = 803)**	**Unselected birth cohort from the same region (n = 747)**
R501X	0.0339 (0.0261-0.044)	0.0248 (0.018-0.0339)
2282del4	0.0296 (0.0223-0.0391)	0.0274 (0.0203-0.0370)
R2447X	0.0069 (0.0039-00124)	0.0033 (0.0014-0.078)
S3247X	0.0044 (0.0021-0.009)	0.0027 (0.0010-0.0069)
3702delG	0.0006 (0.000-0.0036)	0.0007 (0.0000-0.0038)
Combined null genotype	0.0767 (0.0646-0.0909)	0.0589 (0.0481-0.0720)

Using a Fisher exact test to compare allele frequencies between groups, the result was a *P* value of greater than .05 for each of the mutations when analyzed individually and for the combined null genotype, indicating that there is no significant difference in minor allele frequencies between the study group and the unselected birth cohort.