

# Filaggrin mutations, atopic eczema, hay fever, and asthma in children

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**Background:** Mutations in the filaggrin gene (*FLG*) have been shown to play a significant role in ichthyosis vulgaris and eczema, 2 common chronic skin diseases. However, their role in the development of other atopic diseases such as asthma and rhinitis has not yet been clarified in large population-based studies.

**Objectives:** To study the effect of *FLG* mutations at the population level and their effect on other atopic phenotypes.

**Methods:** Association analysis of the 2 common *FLG*-null mutations R501X and 2282del4 and 3 recently identified rare *FLG* variants (R2447X, S3247X, 3702delG) was performed on our cross-sectional population of German children (n = 3099) recruited as part of the International Study of Asthma and Allergies in Childhood II in Munich (n = 1159) and Dresden (n = 1940).

**Results:** *FLG* variants increased the risk for eczema more than 3-fold (odds ratio [OR], 3.12; 95% CI, 2.33-4.173;  $P = 2.5 \times 10^{-14}$ ; population-attributable risk, 13.5%). Independent of eczema, *FLG* mutations conferred a substantial risk for allergic rhinitis (OR, 2.64; 95% CI, 1.76-4.00;  $P = 2.5 \times 10^{-6}$ ; population-attributable risk, 10.8%). Nasal biopsies demonstrated strong filaggrin expression in the cornified epithelium of the nasal vestibular lining, but not the transitional and respiratory nasal epithelia. In contrast, the association with asthma (OR, 1.79; 95% CI, 1.19-2.68;  $P = .0048$ ) was restricted to asthma occurring in the context of eczema, and there was a strong association with the complex phenotype eczema plus asthma (OR, 3.49; 95% CI, 2.00-6.08;  $P = 1.0 \times 10^{-5}$ ).

**Conclusion:** Our results suggest that *FLG* mutations are key organ specific factors predominantly affecting the development of eczema and confer significant risks of allergic sensitization and allergic rhinitis as well as asthma in the context of eczema. (J Allergy Clin Immunol ■■■■;■■■:■■■-■■■.)

**Key words:** Atopic eczema, rhinitis, asthma, filaggrin, children

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Recently a breakthrough in the genetics of atopic eczema was achieved by the identification of 2 mutations (R501X and 2282del4) within the filaggrin gene (*FLG*).<sup>1,2</sup> Both mutations lead to a premature translation stop of the filaggrin protein, resulting in a filaggrin deficiency. Further rare *FLG* mutations reported in Irish subjects have also been shown to be functional null alleles.<sup>3</sup>

A substantial proportion of the white population may have a primary barrier dysfunction caused by a genetic filaggrin deficiency and thereby be prone to developing inflammatory skin diseases.<sup>4</sup> Although *FLG* mutations cause ichthyosis vulgaris, one of the most common inherited cutaneous keratinization disorders, they also represent a major predisposing factor for atopic eczema.<sup>1,2</sup> Independent replication studies have provided unequivocal evidence for the exceptionally strong and consistent effect of *FLG* null alleles on white populations, predisposing particularly to an early-onset, severe, and persistent course of eczema.<sup>5</sup>

These initial family-based and case-control studies were all centered on the effect of *FLG* in patients with eczema, whereas so far, the incidence of *FLG* null alleles in the general population and their role in atopic diseases other than eczema has not been determined sufficiently. The expression of filaggrin in the skin and other keratinizing epithelia such as the oral cavity and the conjunctiva<sup>6-8</sup> may suggest that organs in addition to the skin could be affected by *FLG* mutations.

**Abbreviations used**

EDC: Epidermal differentiation complex

FLG: Filaggrin gene

HWE: Hardy-Weinberg equilibrium

ISAAC II: International Study of Asthma and Allergies in  
Childhood, phase II

OR: Odds ratio

PAR: Population-attributable risk

SPT: Skin prick test

We genotyped 3099 German children from the population-based cross-sectional International Study of Asthma and Atopy in Childhood, phase II (ISAAC II) for 5 *FLG* variants previously described to reach a minor allele frequency of at least 0.01 in a study of Irish subjects<sup>3</sup>: the originally identified mutations in *FLG* repeat 1, R501X and 2282del4, as well as the more 3' mutations R2447X, S3247X, and 3702delG. The role of *FLG* mutations in the manifestation of a range of atopic diseases such as eczema, asthma, and rhinitis was studied. Furthermore, the probability that a person carrying these mutations will develop an atopic disease (penetrance) and the proportion of cases in the population attributable to the mutant *FLG* alleles (population-attributable risk PAR) were determined.

**METHODS****Nomenclature**

The terminology for atopic diseases is confusing, and terms such as *atopic eczema*, *atopic dermatitis*, *childhood eczema*, *atopiform dermatitis*, and *flexural dermatitis* are frequently used synonymously in the literature. In an attempt to standardize the nosology for allergic diseases, the nomenclature committee of the World Allergy Organization recently published its recommendation for naming allergic diseases such as asthma, eczema, and rhinitis.<sup>9</sup> In this article, we try to follow these recommendations, although this study was performed before the World Allergy Organization suggestions.

**Study population and phenotyping**

Between 1995 and 1996, a cross-sectional study was performed in Munich and Dresden, Germany,<sup>10</sup> as part of ISAAC II to assess the prevalence of asthma and allergies in all schoolchildren attending 4th class in both cities (age 9-11 years) using standardized and validated study tools.<sup>10-13</sup> Within the study population of 5629 children, all children of German origin with DNA available from Munich (n = 1159) and Dresden (n = 1940) were included in this analysis (n = 3099).

Skin tests and serum measurements for total and specific IgE were performed according to standardized procedures as previously described.<sup>10</sup> Skin prick tests (SPTs) to 6 common aeroallergens (*Dermatophagoides pteronyssinus*, *Dermatophagoides farinae*, *Alternaria tenuis*, cat dander, mixed grass, and tree pollen, all from ALK-Scherax, Wedel, Germany) were performed. A child was considered atopic if a wheal reaction  $\geq 3$  mm occurred to 1 or more allergens after subtraction of the negative control. Specific IgE antibodies against a panel of aeroallergens (Sx1 CAP from Phadia, Freiburg, Germany) and food allergens (FX5 CAP; Phadia) were measured in a range between 0.35 and 100 IU/mL.

Children whose parents reported a physician's diagnosis of endogenous or atopic dermatitis were classified as having eczema. Children whose parents reported a physician's diagnosis of asthma (at least once) or of spastic bronchitis or recurrent asthmatic bronchitis (at least twice) in a self-administered questionnaire were classified as having asthma. The definition of allergic rhinitis was based on the parent's information of a doctor's diagnosis of hay fever in combination with a positive SPT against at least 1 of the allergens tested.

**TABLE I.** Descriptive characterization of the ISAAC II study population comparing genotyped individuals included in this analysis (N = 3099)

|                                    | No.*      | Percent |
|------------------------------------|-----------|---------|
| Male sex                           | 1561/3099 | 50.4    |
| Age (y), mean (SD)                 | 9.6 (0.6) |         |
| Eczema                             | 540/2994  | 18.0    |
| Atopic eczema                      | 193/2901  | 6.7     |
| Asthma                             | 272/3054  | 8.9     |
| Atopic asthma                      | 124/2906  | 4.3     |
| Allergic rhinitis                  | 214/2938  | 7.3     |
| Sensitization                      | 777/3002  | 25.9    |
| Current wheeze                     | 256/3052  | 8.4     |
| Currently visible eczema           | 169/3098  | 5.5     |
| Current allergic rhinitis symptoms | 441/3037  | 14.5    |

\*Number affected/number with data available.

Eczema and asthma were divided into atopic and nonatopic on the basis of positive SPTs against at least 1 of the allergens tested. *Current allergic rhinitis symptoms* was defined as the parent's report of an itchy runny nose and sneezing in the last 12 months in the absence of a cold.

The following subphenotypes of eczema were analyzed: *visible eczema*, defined according to standardized and validated criteria at inspection determined by a trained pediatrician<sup>14</sup>; and *current eczema symptoms*, defined as parent's report of the presence of an itchy rash in the last 12 months that had affected the skin creases.

Written informed consent was obtained from all parents of children included in the study. All study methods were approved by ethics committees of the medical faculty of the University of Münster (ISAAC phase II data center) and the ethics committee of the Bavarian medical council (center for genetic analysis).

**Genotyping**

Genomic DNA was extracted from whole blood by a standard salting out method.<sup>15</sup> To increase DNA yield, random DNA preamplification with GenomiPhi (Amersham Biosciences, Freiberg, Germany) was performed. DNA samples were genotyped for R501X, 2282del4, and 3702delG using matrix-assisted laser desorption/ionization time-of flight mass spectrometry ([www.sequenom.com](http://www.sequenom.com)). PCR assays and associated extension reactions were designed using the SpectroDESIGNER software (Sequenom, San Diego, Calif). All amplification and extension reaction conditions have been previously described.<sup>16</sup> Primer extension products were analyzed by a MassARRAY mass spectrometer (Sequenom; [www.bdal.de](http://www.bdal.de)), and resulting mass spectra were analyzed using the SpectroTYPER RT 2.0 software (Sequenom). Primers for genotyping were ACGTTGGATGCTGGAGGAAGACAAGGA TCG, ACGTTGGATGATGGTGTCTGA CCCTCTTG, and ATGCCTGG AGCTGTCTC (extension primer) for R501X; ACGTTGGATGTCTTGG TGGCTCTGCTGATG, ACGTTGGATGGGGACATTCAGAA GACTCAG, and GACTCAGACACACAGT (extension primer) for 2282del4; and ACGTT GGATGTGTGAGTGTCTAGAGCTGTC, ACGTTGGATGACAAACAATC AGG AGACGGC, and GCTCCAGGCACTCAGGT (extension primer) for the 3702delG variant. Genotyping of R2447X and S3247X was performed by using the TaqMan allelic discrimination method (Applied Biosystems, Foster City, Calif), and the 3702delG genotyping was repeated using this method as also reported by Sandilands et al.<sup>3</sup> To control for errors and technical problems in genotyping, derived genotype frequencies were compared with the expected allelic population equilibrium based on the Hardy-Weinberg equilibrium (HWE) test.

**Histology**

Normal nasal tissue in 4- $\mu$ m sections was obtained at excision of an unrelated dermal-based mesenchymal lesion from an otherwise healthy patient.

TABLE II. Frequency of *FLG* null alleles R501X, 2282del4, R2447X, and S3247X\* in children affected by eczema or allergic rhinitis and unaffected children†‡

|    | R501X    |            |         |                   |            |         | 2282del4 |            |         |                   |            |          | R2447X   |            |         |                   |            |         | S3247X   |            |         |                   |            |         |
|----|----------|------------|---------|-------------------|------------|---------|----------|------------|---------|-------------------|------------|----------|----------|------------|---------|-------------------|------------|---------|----------|------------|---------|-------------------|------------|---------|
|    | Eczema   |            |         | Allergic rhinitis |            |         | Eczema   |            |         | Allergic rhinitis |            |          | Eczema   |            |         | Allergic rhinitis |            |         | Eczema   |            |         | Allergic rhinitis |            |         |
|    | Affected | Unaffected |         | Affected          | Unaffected |         | Affected | Unaffected |         | Affected          | Unaffected |          | Affected | Unaffected |         | Affected          | Unaffected |         | Affected | Unaffected |         | Affected          | Unaffected |         |
| AA | 511      | 2395       | (94.6%) | 200               | 2650       | (93.5%) | 479      | 2294       | (88.7%) | 190               | 2537       | (93.1%)  | 519      | 2386       | (97.2%) | 207               | 2645       | (97.1%) | 525      | 2362       | (96.3%) | 207               | 2627       | (96.4%) |
| Aa | 25       | 32         | (4.6%)  | 11                | 48         | (5.1%)  | 52       | 86         | (9.6%)  | 21                | 31         | (4.0%)   | 10       | 10         | (1.9%)  | 1                 | 19         | (0.7%)  | —        | 6          | (0.2%)  | —                 | 5          | (0.2%)  |
| Aa | —        | —          | —       | —                 | —          | —       | —        | 1          | (0.04%) | —                 | 1          | (0.004%) | —        | —          | (0.4%)  | —                 | —          | (0.7%)  | —        | —          | (0.2%)  | —                 | 1          | (0.04%) |

For combined genotypes of R501X, 2282del4, R2447X, and S3247X variants, the minor allele label *a* refers to the dominant mutant alleles.

\*No carriers of 3702G observed.

†Unaffected children are defined as individuals without the investigated trait.

‡Percentages are given with respect to 3099 children included in the study. Individuals with missing genotype or phenotype information are not explicitly shown in the table.

Multiple sections for immunostaining were taken at regular intervals along a continuum from the free alar margin through transitional epithelium and nasal respiratory epithelium. Immunohistochemical staining was performed by using the Biogenesis antifilaggrin antibody (Biogenesis, Poole, UK) (catalog no. 4480-0905) after automated antigen retrieval by heat on the Benchmark automated stainer (Ventana Medical Systems, Tucson, Ariz).

## Statistical analysis

Descriptive statistics for quantitative values are given as means ( $\pm$ SDs) and for qualitative values by absolute and relative frequencies. To test the fit of genotype frequencies with the HWE, a  $\chi^2$  goodness-of-fit test was used, implemented in PROC ALLELE (SAS statistical software package 9.1; SAS Institute, Cary, NC). Association of binary traits with genetic variations and thus estimation of the odds ratios (ORs) with 95% CIs was evaluated by logistic regression models using SAS 9.1. Models were adjusted for sex, and best parameter sets to fit the model were found by using backward selection methods. In a secondary approach, based on the known genetic effect of *FLG* mutations, eczema was specified as an additional covariate to evaluate genetic effects on binary traits independently of eczema. Values for total serum IgE levels were first log-transformed, and linear regression models were applied. Skin test results were analyzed by using logistic models. The population attributable risk (PAR) was calculated as follows:  $PAR = [Prev_E (OR-1)]/[1 + Prev_E (OR-1)]$ , where  $Prev_E$  is the prevalence of exposure (proportion of *FLG* mutation carriers in the population) and OR is the estimated OR from multiple regression models.<sup>17</sup>

## RESULTS

The clinical characteristics of the study population are outlined in Table I. For the current study, 5 *FLG* mutations previously identified to result in a deficiency of filaggrin expression were genotyped in 3099 German children. The genotyping success rate was 97.3% for 2282del4, 98.9% for R501X, 97.7% for R2447X, 96.7% for S3247X, and 97.0% for 3702delG. Carriers of 3702delG could be identified with neither matrix-assisted laser desorption/ionization time-of flight nor TaqMan genotyping in our population. Observed genotype frequencies did not deviate significantly from expected frequencies under the HWE assumption.

In this cross-sectional German population, the 2282del4 variant was present in 4.6% of children, and the frequency of carriers of R501X was 1.9%, with 0.1% of children carrying both mutations concomitantly, resulting in a combined carrier frequency of 6.4%. R2447X was present in 0.7% and S3247X in 0.2% of children. The combined carrier frequency of R501X, 2282del4, R2447X, and S3247X was 7.4% (Tables II and III).

Table IV summarizes the results of association analysis. *FLG* variants were strongly overrepresented in eczema cases (OR, 3.12; 95% CI, 2.33-4.17;  $P = 2.5 \times 10^{-14}$ ) for combined genotype). In addition, in the total population we observed a significantly increased risk of rhinitis (OR, 2.32; 95% CI, 1.59-3.36;  $P = 1.0 \times 10^{-5}$ ) and asthma (OR, 1.79; 95% CI, 1.19-2.68;  $P = .0048$ ). Associations became even stronger when restricting the analysis to the allergic subphenotypes atopic eczema (OR, 4.57; 95% CI, 3.08-6.74;  $P = 3.2 \times 10^{-14}$ ) and allergic rhinitis (OR, 2.64; 95% CI, 1.76-3.96;  $P = 2.5 \times 10^{-6}$ ). Independent analysis of the Munich ( $n = 1159$ ) and Dresden ( $n = 1940$ ) populations showed associations with rhinitis and allergic rhinitis in both cohorts (see this article's Table E1 in the Online Repository at [www.jacionline.org](http://www.jacionline.org)). Accordingly, *FLG* alleles conferred an increased risk for sensitization as measured by skin test (OR, 1.61; 95% CI, 1.20-2.17;  $P = .0017$ ), which was strongest in individuals

**TABLE III.** Frequency of combined common *FLG* null alleles in children affected by eczema or allergic rhinitis and unaffected children\*†

|    | Combined genotype (R501X, 2282del4, R2447X, S3247X) |              |                   |              |
|----|-----------------------------------------------------|--------------|-------------------|--------------|
|    | Eczema                                              |              | Allergic rhinitis |              |
|    | Affected                                            | Unaffected   | Affected          | Unaffected   |
| AA | 437 (80.9%)                                         | 2206 (89.9%) | 175 (81.8%)       | 2420 (88.8%) |
| Aa | 83 (15.4%)                                          | 134 (5.5%)   | 33 (15.4%)        | 178 (6.5%)   |
| aa | 2 (0.4%)                                            | 2 (0.08%)    | —                 | 4 (0.2%)     |

For combined genotypes of R501X, 2282del4, R2447X, and S3247X variants, the minor allele label *a* refers to the dominant mutant alleles.

\*Unaffected children are defined as individuals without the investigated trait.

†Percentages are given with respect to 3099 children included in the study. Individuals with missing genotype or phenotype information are not explicitly shown in the table.

**TABLE IV.** ORs and 95% CIs for associations between *FLG* mutations and selected atopic phenotypes based on a logistic regression models adjusted for sex by using backward variable selection (OR calculated for heterozygote vs wild-type)

| Phenotype                                    | Combined genotype (R501X, 2282del4, R2447X, S3247X) |        |       |                       |
|----------------------------------------------|-----------------------------------------------------|--------|-------|-----------------------|
|                                              | OR                                                  | 95% CI |       | P value               |
| Eczema                                       | 3.115                                               | 2.326  | 4.173 | $2.5 \times 10^{-14}$ |
| Atopic eczema                                | 4.556                                               | 3.080  | 6.740 | $3.2 \times 10^{-14}$ |
| Currently visible eczema                     | 2.981                                               | 1.954  | 4.548 | $4.0 \times 10^{-7}$  |
| Asthma                                       | 1.790                                               | 1.194  | 2.684 | .0048                 |
| Atopic asthma                                | 1.777                                               | 0.994  | 3.175 | .0524                 |
| Current wheeze                               | 1.752                                               | 1.156  | 2.655 | .0082                 |
| Allergic rhinitis                            | 2.640                                               | 1.762  | 3.955 | $2.5 \times 10^{-6}$  |
| Current allergic rhinitis symptoms           | 1.686                                               | 1.200  | 2.371 | .0026                 |
| Sensitization (skin test reactivity)         | 1.609                                               | 1.195  | 2.167 | .0017                 |
| Sensitization adjusted for eczema            | 1.460                                               | 1.073  | 1.986 | .0160                 |
| Allergic rhinitis adjusted for eczema        | 2.245                                               | 1.480  | 3.406 | .0001                 |
| Allergic rhinitis adjusted for atopic eczema | 1.925                                               | 1.214  | 3.053 | .0053                 |
| Asthma adjusted for eczema                   | 1.515                                               | 0.999  | 2.299 | NS                    |
| Asthma adjusted for atopic eczema            | 1.375                                               | 0.851  | 2.222 | NS                    |
| Atopic asthma adjusted for eczema            | 1.379                                               | 0.757  | 2.514 | NS                    |
| Atopic asthma adjusted for atopic eczema     | 1.111                                               | 0.586  | 2.107 | NS                    |

**TABLE V.** Association of *FLG* mutations with selected atopic phenotypes in the presence and absence of eczema

|                      | Allergic rhinitis |        |       |         | Asthma |        |       |         | Atopic asthma |        |       |         |
|----------------------|-------------------|--------|-------|---------|--------|--------|-------|---------|---------------|--------|-------|---------|
|                      | OR                | 95% CI |       | P value | OR     | 95% CI |       | P value | OR            | 95% CI |       | P value |
| Eczema (n = 540)     | 2.001             | 1.078  | 3.176 | .0280   | 1.518  | 0.831  | 2.773 | NS      | 1.646         | 0.766  | 3.539 | NS      |
| No eczema (n = 2454) | 2.471             | 1.415  | 4.316 | .0015   | 1.525  | 0.855  | 2.721 | NS      | 1.046         | 0.374  | 2.927 | NS      |

with eczema (OR, 2.04; 95% CI, 1.25-3.33;  $P = .0043$ ). Similar trends were observed for specific serum IgE levels (data not shown). The association with atopic asthma *per se* was only borderline significant (OR, 1.78; 95% CI, 0.99-3.18;  $P = .0524$ ), whereas the complex asthma plus eczema phenotype was strongly associated (OR, 3.49; 95% CI, 2.00-6.08;  $P = 1.0 \times 10^{-5}$ ). After adjusting for eczema or atopic eczema, associations with asthma and atopic asthma still showed a positive trend, but the association was now no longer significant. In contrast, the association with allergic rhinitis (OR, 2.25; 95% CI, 1.48-3.41;  $P = 1.0 \times 10^{-4}$ ; and OR, 1.93; 95% CI, 1.21-3.05;  $P = .0053$ ) remained significant (Table IV). Also, an analysis stratified for eczema showed associations with rhinitis but not with asthma (Table V). However, the association with current allergic rhinitis symptoms was clearly weaker after adjustment for eczema (OR, 1.40; 95% CI, 0.99-2.00;  $P = .061$ ). Additional analyses of subphenotypes showed strong associations of asthma plus rhinitis (OR, 2.63; 95% CI, 1.21-5.70) and rhinitis but no asthma (OR, 2.54; 95%

CI, 1.62-4.00), whereas the association of asthma but no rhinitis was only borderline significant (OR, 1.53; 95% CI, 0.941-2.495).

Immunohistochemical staining of skin in multiple, serially taken sections in the nasal vestibule up to the transitional epithelium consistently showed a typical granular pattern of filaggrin staining in the granular layer and a more diffuse pattern in the stratum corneum, consistent with normally differentiated hair-bearing skin. No filaggrin expression was seen in the transitional mucosa or respiratory mucosa (Fig 1).

For eczema, the penetrance of *FLG* null alleles was 38.5%, and the PAR, which indicates the proportion of eczema cases in the population attributable to the mutant *FLG* alleles, was 13.5% on the basis of the observed carrier frequency of mutant *FLG* alleles of 7.4% in the population. For allergic rhinitis, the PAR was 10.8%, and 8.4% when adjusted for eczema, whereas the PAR for allergic rhinitis in combination with eczema was as high as 20.1% and for asthma in combination with eczema was 15.6% (Table VI).

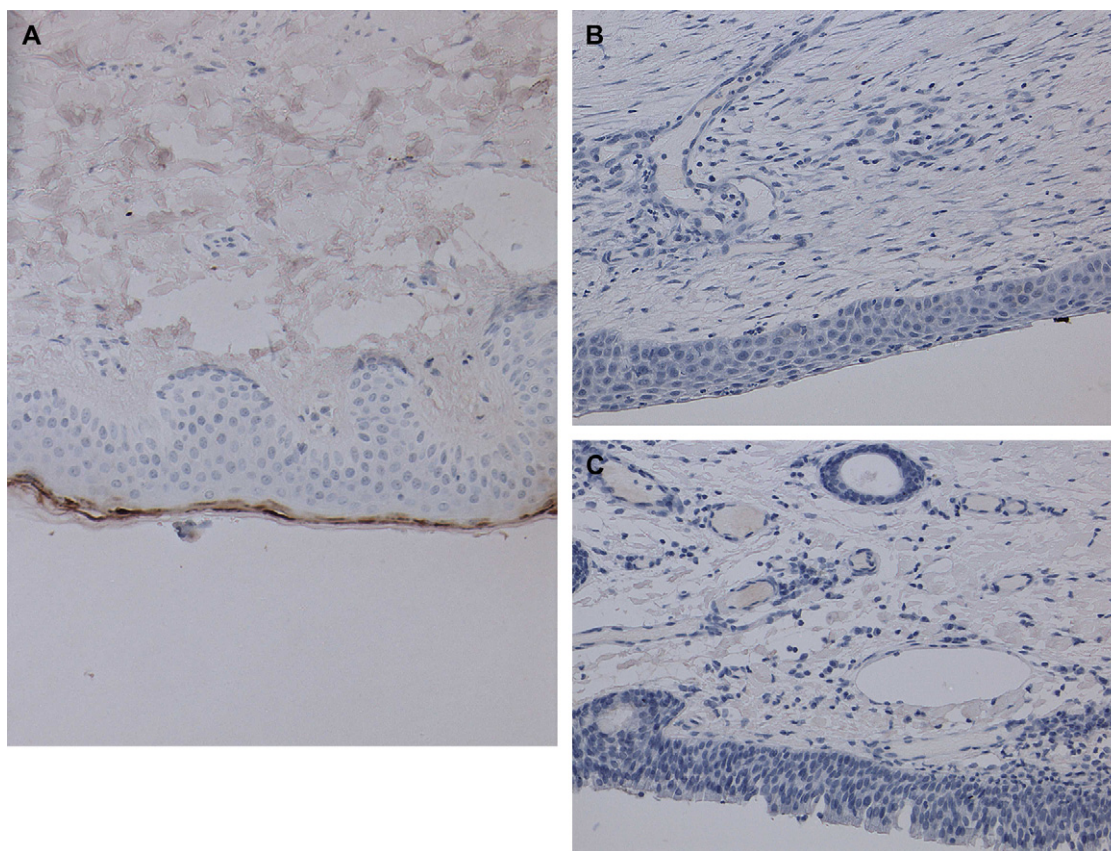


**FIG 1.** Nasal filaggrin expression. Sections of vestibular skin (A), transitional epithelium (B), and respiratory mucosa (C). In the vestibular skin, staining is identical to the pattern observed in normal hair-bearing skin with strong granular staining in the granular layer and more diffuse staining of the stratum corneum. In transitional and respiratory nasal epithelia, no typical filaggrin staining is demonstrable. There is minimal edge artefact that is not significant. All original magnifications  $\times 100$ .

## DISCUSSION

This is a large-scale population-based study dissecting the influence of *FLG* mutations on eczema and a range of atopic diseases such as asthma and allergic rhinitis in the general population. In addition to exceptionally strong and consistent effects of *FLG* mutations on eczema and atopic eczema, associations with allergic sensitization and allergic rhinitis, independently of eczema, were demonstrated. In contrast, and in line with previous studies,<sup>2,18,19</sup> an association with asthma was noted, but there was no direct effect of *FLG* mutations on asthma or atopic asthma in the absence of eczema. However, as also observed in the large longitudinal population-based Avon Longitudinal Study of Parents and Children, there was a strong association with the complex asthma plus eczema phenotype with an OR of 3.49 in ISAAC II (OR for asthma plus eczema, 3.42 in ALSPAC).<sup>20</sup>

On the basis of these results, *FLG* must be considered to be a major gene in the development of eczema, atopic sensitization, and allergic rhinitis, with a significant effect in asthma that is restricted to patients with a history of eczema.

Filaggrin is a key protein in the formation of the outermost keratin layer of the skin,<sup>21</sup> with additional properties contributing to the hydration of the stratum corneum.<sup>22</sup> Thus, a genetically determined filaggrin deficiency was first considered and confirmed to be involved in ichthyosis vulgaris, a common disease leading to an excessive scaling of the skin, and in eczema.<sup>1</sup> Concerning

clinically manifest eczema, *FLG*-null alleles show an incomplete penetrance of 38.5%. Recently we demonstrated a strong association of *FLG* mutations with dry skin as an intermediate trait.<sup>23</sup> Thus, it seems plausible that *FLG* mutations primarily cause a dry or defective skin barrier which, when driven by additional genetic and environmental factors, results in eczema in a large proportion of carriers.

Independently from eczema, *FLG* mutations showed a significant association with allergic sensitization and allergic rhinitis in our population. The association with allergic rhinitis was also independent from the presence (OR, 2.63; 95% CI, 1.21-5.70) or absence (OR, 2.54; 95% CI, 1.62-4.00) of concomitant asthma, and could be observed in both subpopulations when analyzed independently (data not shown). In contrast, the contribution of *FLG* mutations to the complex etiology of asthma *per se* seems to be limited to those with previous eczema.

Our results support recent epidemiologic data indicating an early comanifestation of atopic eczema and asthma rather than a progressive atopic march<sup>24</sup> and a stronger overlap between eczema and allergic rhinitis.<sup>25,26</sup> However, in contrast with allergic rhinitis, the observed risk for the trait current allergic rhinitis symptoms (reflecting the 12-month period prevalence of symptoms before recruitment) was only borderline significant after adjustment for eczema. This observation is in line with findings from the ALSPAC cohort, in which, in contrast with our study,

**TABLE VI.** PAR estimates and 95% CIs and penetrance (with exact *P* value) caused by *FLG* mutations R501X, 2282del4, R2447X, and S3247X (based on the observed carrier frequency for the combined genotype of 7.4%)

|                            | PAR   | 95% CI |       | Penetrance                      |
|----------------------------|-------|--------|-------|---------------------------------|
| Eczema                     | 13.5% | 8.9%   | 19.0% | 38.5% ( $1.3 \times 10^{-13}$ ) |
| Allergic rhinitis          | 10.8% | 5.3%   | 17.9% | 15.4% ( $3.1 \times 10^{-5}$ )  |
| Eczema + allergic rhinitis | 20.1% | 9.9%   | 33.4% | 8.1% ( $9.7 \times 10^{-6}$ )   |
| Eczema + asthma            | 15.6% | 6.9%   | 27.3% | 8.3% ( $3.2 \times 10^{-5}$ )   |

*FLG* associations with allergic rhinitis were limited to those with previous eczema.<sup>20</sup> In this context, it has to be considered that our study is a cross-sectional study that documents the precise and detailed phenotypes present in the study population at the time point when they are examined (in this case, 9–11 years of age) and records details of previous illnesses (physician-diagnosed eczema ever) as recalled by the parents at the time of interview. Thus, there is a possibility of recall bias against previous eczema, and it cannot be ruled out that the effect of filaggrin mutations on allergic rhinitis observed here in 2 independent populations was entirely independent of previous eczema and that *FLG* mutations exert an effect on rhinitis as a result of previous eczema, however mild. Carefully phenotyped studies will be needed to clarify these open questions.

Filaggrin is expressed in other keratinizing epithelia—for example, in the oral cavity<sup>7</sup> and the ocular conjunctiva.<sup>6</sup> So far, filaggrin has not been detected in the human bronchial epithelium.<sup>8,27</sup> Here, we demonstrate that filaggrin is expressed in the anterior vestibulum of the nose, but not in transitional and respiratory nasal epithelia. Thus, it seems unlikely that *FLG* mutations exert organ-specific and local effects in the upper airways. Indeed, the mechanisms through which *FLG* mutations contribute to airway disease are not understood yet. Percutaneous priming<sup>28</sup> and secondary immunologic effects from the induction of T<sub>H</sub>2 cytokines in epithelia<sup>29</sup> are interesting hypotheses that have been put forward, but need further investigation.

*FLG* mutations are strongly overrepresented in children with atopic eczema or allergic rhinitis as indicated in Tables II and III. Because of the low frequencies of minor alleles of 2.40% for 2282del4 and 0.96% for R501X, individuals homozygote for the mutations are rarely found, even after genotyping 3099 individuals. Interestingly, 2 out of 3 individuals carrying 2 mutations have eczema, whereas none of them seems to be affected by rhinitis at the age of 9 to 11 years, when the survey was conducted, demonstrating that genotype-phenotype correlation is not completely linear and the penetrance of the *FLG* effect is incomplete. Although these preliminary results are intriguing, caution should be used when drawing conclusions based on the very small numbers of homozygotes seen here.

However, with the estimated penetrance of 38.5% and a PAR of 13.5% for eczema and 10.8% for allergic rhinitis as well as 20.1% and 15.6% for the complex phenotypes eczema plus rhinitis and eczema plus asthma, respectively, the effect of these single gene variants is extremely strong. The PAR for eczema plus asthma is very consistent with the ALSPAC cohort, which estimated this to be 15.6%.<sup>20</sup> Although previously only rough estimates of PAR based on the 2 most common variants existed,<sup>18</sup> this is the first assessment of the PAR truly based on data from the general population and including less frequent variants. Interestingly, both the originally identified null mutations (R501X and 2282del4) and the less common variants R2447X, S3247X, and 3702delG

appear to be significantly more prevalent in United Kingdom and Irish populations (compared with continental Europe),<sup>1,3,30</sup> which might reflect ethnic differences. Furthermore, the genetic architecture of *FLG*-related eczema mutations seems to consist of a mixture of prevalent *FLG* mutations common to different populations and several infrequent, family-specific, and private mutations.<sup>31</sup>

The *FLG* gene is located within the epidermal differentiation complex (EDC) on chromosome 1q21. The EDC is a dense cluster of genes involved in the terminal differentiation of the epidermis and formation of the stratum corneum.<sup>32</sup> Because the EDC comprises further genes and gene families important for the differentiation of the human epidermis, it is conceivable that additional genetic variants in other genes of the EDC may additionally contribute to the risk to develop eczema.<sup>33</sup> Whether any of these genes may also be involved in the development of airway disease remains to be seen.

Our results may help us understand better the genetic susceptibility of complex diseases. The strong impact of *FLG* mutations with a rather low frequency on eczema and allergic rhinitis challenges the common disease-common variants hypothesis suggesting that such common diseases as asthma, eczema, and rhinitis are predominantly caused by mutations highly frequent in the population.<sup>34,35</sup> The fact that infrequent mutations may be associated with common diseases needs consideration, because a new generation of genome-wide association studies is emerging, predominantly based on frequent haplotype-tagging polymorphisms. Using these approaches, *FLG* effects would have been missed.

In addition, the “1 airway, 1 disease” hypothesis suggesting that the nose and the lung share the same mucosa and that therefore the same mechanisms lead to the development of rhinitis and asthma, might be an oversimplification.<sup>36,37</sup>

Now that *FLG* mutations have been established as a major cause of atopic eczema, their newly discovered role in the development of allergic rhinitis needs to be further elucidated, including replication of associations and systematic expression studies in the upper and lower airways. Additional large-scale and longitudinal studies will be needed to give definite answers about how *FLG* mutations may affect the development of asthma. Furthermore, ethnic differences in allele frequencies across Europe and the world need to be studied.

Assessing the effects of *FLG* deficiency has been the first step toward the understanding of a major disease mechanism in the development of eczema, asthma, and allergic rhinitis. Reconstitution of filaggrin function or compensation of filaggrin deficiency may be next steps building the groundwork for new strategies in prevention and treatment of these common diseases.

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**Clinical implications: *FLG* mutations predispose to eczema and are also risk factors for allergic sensitization and allergic rhinitis. Patients with filaggrin-related eczema are at increased risk of developing asthma.**

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**TABLE E1.** Association of *filaggrin* mutations with allergic rhinitis in ISAAC II

| Phenotype                                                                       | Combined genotype (R501X, 2282del4, R2447X, S3247X) |        |       |                      |
|---------------------------------------------------------------------------------|-----------------------------------------------------|--------|-------|----------------------|
|                                                                                 | OR                                                  | 95% CI |       | P value              |
| Physician-diagnosed allergic rhinitis (Munich and Dresden combined)             | 2.320                                               | 1.598  | 3.368 | $1.0 \times 10^{-5}$ |
| Physician-diagnosed allergic rhinitis Munich                                    | 3.603                                               | 2.012  | 6.453 | $1.6 \times 10^{-5}$ |
| Physician-diagnosed allergic rhinitis Dresden                                   | 1.765                                               | 1.079  | 2.888 | .0238                |
| Allergic rhinitis (physician's diagnosis and SPT reaction to inhalant allergen) | 2.640                                               | 1.762  | 3.955 | $2.5 \times 10^{-6}$ |
| Allergic rhinitis Munich                                                        | 4.065                                               | 2.199  | 7.514 | $7.7 \times 10^{-6}$ |
| Allergic rhinitis Dresden                                                       | 1.975                                               | 1.145  | 3.407 | .0144                |
| Current allergic rhinitis symptoms (last 12 mo)                                 | 1.686                                               | 1.200  | 2.371 | .0026                |
| Current allergic rhinitis symptoms Munich                                       | 1.850                                               | 1.094  | 3.128 | .0217                |
| Current allergic rhinitis symptoms Dresden                                      | 1.571                                               | 1.002  | 2.463 | .0491                |

Combined and independent analyses of filaggrin associations with physician-diagnosed allergic rhinitis, without/in combination with allergic sensitization to inhalant allergens and symptoms of allergic rhinitis within the last 12 months (current symptoms) in the Munich (n = 1159) and Dresden (n = 1940) samples.