

Meta-analysis of filaggrin polymorphisms in eczema and asthma: Robust risk factors in atopic disease

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Background: The discovery of filaggrin (*FLG*) null mutations as a major risk factor for eczema represents a milestone toward the understanding of an important mechanism in this complex disease. However, published studies demonstrate differences concerning design and effect size, and conflicting results for asthma have been reported.

Objectives: We sought to provide a more accurate estimate of *FLG* effect sizes and to better refine *FLG* risk profiles within the broad and inclusive eczema diagnosis. We also sought to provide a more detailed and conclusive estimate of the risk for asthma associated with *FLG* null alleles.

Methods: We performed a meta-analysis of 24 studies on *FLG* mutations and eczema involving 5,791 cases, 26,454 control subjects, and 1,951 families as well as 17 studies on asthma involving 3,138 cases, 17,164 control subjects, and 1,511 offspring. **Results:** Both case-control and family studies showed strong associations with eczema. Case-control studies were heterogeneous, whereas family studies yielded more homogeneous results. Combined analysis showed that *FLG* haploinsufficiency strongly increases the eczema risk (odds ratio

[OR], 3.12; 95% CI, 2.57-3.79) and is associated with more severe and dermatologist-diagnosed disease. *FLG* mutations are also significantly associated with asthma (OR, 1.48; 95% CI, 1.32-1.66). However, although strong effects for the compound phenotype asthma plus eczema (OR, 3.29; 95% CI, 2.84-3.82) were observed, there appears to be no association with asthma in the absence of eczema.

Conclusions: This meta-analysis summarizes the strong evidence for a high eczema risk conferred by *FLG* mutations and refines their risk profiles, suggesting an association with more severe and secondary care disease. *FLG* mutations are also a robust risk factor for asthma and might help define the asthma endophenotype linked with eczema. (J Allergy Clin Immunol 2009;123:1361-70.)

Key words: Atopic eczema, atopic dermatitis, eczema, asthma, filaggrin, meta-analysis

(Atopic) eczema (atopic dermatitis) is the most common chronic inflammatory skin disease and affects up to 30% of children and 10% of adults in developed societies. A considerable percentage of patients with eczema (especially those with moderate or severe disease in secondary care settings) exhibit increased total IgE levels and specific sensitizations to environmental allergens, and eczema often predates the development of allergic airway disease.¹

Atopic disorders are strongly genetic. Based on the assumption that these diseases are primarily immunologic in etiopathogenesis, for many years genetic research has focused on genes associated with immunologic abnormalities.² The recent identification of mutations in the gene encoding the stratum corneum structural protein filaggrin (*FLG*) as a remarkably strong and widely replicated risk factor for eczema,^{3,4} however, has led to a paradigm shift and has placed an inherited disturbance of the epidermal barrier into the center of pathogenetic concepts.¹

To date, 2 common *FLG* variants (R501X and 2282del4) with an estimated combined allele frequency of approximately 6%, as well as 18 less common variants, all in trans with R501X and 2282del4, have been identified in individuals of European descent. An additional 17 mutations appear to be restricted to individuals of Asian descent. Each of these reported variants lead to nonsense mutations, such as frameshifts or premature stop codons, and either prevent or severely diminish the production of free *FLG* in the epidermis.⁴

Although *FLG* mutations have been consistently associated with eczema, their role in predisposition to asthma remains a matter of debate.^{5,6} In a meta-analysis of 9 studies that had been conducted until March 2007, we observed an overall odds ratio (OR) of 4.09 from eczema case-control studies and an OR of 2.10 from eczema family studies, with substantial heterogeneity among the

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Abbreviations used

FLG: Filaggrin

OR: Odds ratio

PAR: Population-attributable risk

studies.⁷ Since then, a large number of additional studies have been published, including investigations on large population-based cohorts and in subjects primarily ascertained through a diagnosis of asthma. To assess the range of *FLG* risk on eczema and to provide a more accurate estimate of the effect size, we report the results of an updated meta-analysis based on 24 studies involving 5,791 eczema cases and 26,454 control subjects, as well as 1,951 eczema families with not less than 3,110 affected offspring. To improve appropriate interpretation, we also assessed heterogeneity and clustered studies according to their study characteristics in subanalyses. Furthermore, we tried to better define the contribution of *FLG* variants to asthma in separate analyses of 3,138 patients with asthma and 17,164 control subjects, as well as 4 family studies including 1,511 affected offspring.

METHODS**Data extraction and abstraction**

The extant nosology of atopic disease is confusing, and terms such as *eczema*, *atopic eczema*, 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 Following this definition, the term *eczema* includes the disease formerly called *atopic eczema* or *atopic dermatitis*, as well as *nonatopic eczema*, whereas the term *atopic eczema* is reserved for those patients with eczema and evidence of IgE involvement. It has to be considered that thus far most studies on *FLG* were performed with DNA collections that had been assembled by using older definitions without distinction between atopic and nonatopic eczema. Analyses presented in this article generally comply with the World Allergy Organization recommendations.

Four researchers (ER, EH, HB, and SW) independently identified published studies and abstracts using the search terms "atopic eczema," "atopic dermatitis," "eczema," "asthma," "FLG," "filaggrin," "R501X," and "2282del4" and their combinations in the databases MEDLINE (National Library of Medicine, Bethesda, Md, 1966-2008) and the ISI Web of Knowledge—Web of Science from the first reported association⁹ up to now (April 2006- January 2009). The highest number of items was obtained by using a combination of the terms "eczema/asthma" and "filaggrin," which resulted in 35 studies retrieved from PubMed and 54 from the ISI Web of Knowledge (date of research: November 5, 2008). Different combinations of the remaining search terms did not produce any new results. Double hits, review articles, and publications without data on the effect of the genetic variations on eczema risk or asthma risk or those only providing data in Asian populations were excluded. This resulted in a final list of 26 articles. Two more publications were excluded^{10,11} because they did not present original data or estimates of the ORs (including 95% CIs) of family, case-control, or population-based studies on *FLG* and eczema or *FLG* and asthma. The reference lists of all retrieved articles were also reviewed to identify publications on the same topics, which did not reveal additional studies.

The following information was abstracted from each study: study design, country of participants, definition and numbers of cases and control subjects, severity of eczema in cases, frequency of genotypes, and consistency of genotype frequencies with Hardy-Weinberg equilibrium. All studies taken into account presented preferably estimated ORs uncorrected for potential confounders or provided complete contingency tables. To broaden the analysis, we supplemented these studies with adjusted ORs if unadjusted estimates were not provided. We communicated with the primary investigators

to obtain clarifications and additional data from their studies when these were not clear in the original publications.

Statistical analysis

In addition to single-mutation analysis of R501X and 2282del4 and additional less common variants in some studies,¹²⁻¹⁵ association analysis was also performed on a combined genotype because the variants are in trans and are considered to have biologically equivalent effects.¹² Individuals carrying 1 mutated and 1 wild-type allele were coded as heterozygous and individuals heterozygous for 2 polymorphisms or homozygous for 1 polymorphism were coded as homozygous for the combined genotype.

Initially, we accounted for different study types and evaluated an overall estimate for case-control and family studies separately. For pooling both study types, we used the allelic inheritance model. Following the method of Risch and Merikangas¹⁶ and defining the 2 alleles as A and B, with B being the mutant variant, we let the risk for individuals of genotype AB be θ times the risk for individuals with genotype AA, a genotype relative risk of θ . To facilitate grouped analysis of several family and case-control studies, we assumed a multiplicative relation for 2 B alleles, and thus the genotype relative risk for genotype BB is θ^2 . Hence for studies that present a 3×2 contingency table for the 3 possible genotypes for cases and control subjects, we coded the respective SNP covariates as 0 for AA, 1 for AB, and 2 for BB and applied a logistic model for estimating the OR and its respective 95% CI. Because in the study by Giardina et al¹⁷ no mutation carrier (AB genotype and BB genotype) was detected in the control group, the estimation would have generated inappropriate values. Therefore we calculated ORs and 95% CIs from the 2×2 table with 0.5 added to each cell.¹⁸

Studies that only provided estimates for ORs and 95% CIs were reviewed for the genetic model, and if the allelic model was not used, we asked the authors to provide estimates using an allelic model or supply the 3×2 contingency table.

In family studies the probability of a heterozygote parent to transmit the possible high-risk B allele is as follows: $P = \theta/(1+\theta)$.¹⁶ Thus $1-P = 1/(1+\theta)$ is the inverse probability, and therefore $P/(1-P) = \theta$, which equals the ratio of transmitted B and untransmitted A (T) to untransmitted B and transmitted A (U) alleles. This can be interpreted as an approximate OR as follows¹⁹: $OR = \frac{T}{U}$.

T can be considered a binomial variable if conditioned on the sum of the numbers of "discordant" pairs (T + U).¹⁹ Thus with the Δ method, we derived a symmetric confidence interval for $\log(OR)$ in family studies.

In case-control and family studies we estimated the allelic risk θ , interpreting this as an OR. Hence it is possible to combine both study types to estimate an overall OR when using logistic regression with appropriate coding of genotype variables²⁰ or by converting the table of genotype counts to allele counts²¹ in case-control studies. In support of this methodological analysis, Knapp et al²² showed that a haplotype relative risk estimate obtained from nuclear families with a single affected child is not larger than an increased relative risk estimate from case-control studies, assuming positive linkage disequilibrium between the marker and disease locus.

For combining the study results, we estimated the overall effect of *FLG* null alleles with a random-effects model.²³ The test of homogeneity was performed according to the method of Cooper and Hedges.²⁴ When the symmetry assumption held, funnel plots were constructed and the test for asymmetry by Egger et al²⁵ was applied to detect a potential publication bias.

The main results of meta-analyses are visualized graphically in forest and funnel plots. Forest plots show the estimated ORs and 95% CIs for risk of atopic eczema and asthma imparted by *FLG* variations for individual studies, group analyses, and combined analyses of all groups. Funnel plots might give a hint as to potential publication bias. If no publication bias exists, then the results of the individual studies are distributed symmetrically and funnel shaped around the overall estimate.

For all analyses, we used an Excel to R Interface for meta-analysis,²⁶ which uses the base, stats, and meta packages in R 2.6.0.²⁷

TABLE I. Association studies of *FLG* polymorphisms and eczema that met the inclusion criteria

Study	CC									FAM								
	R501X			2282del4			Combined			R501X			2282del4			Combined		
	OR	95% CI		OR	95% CI		OR	95% CI		OR	95% CI		OR	95% CI		OR	95% CI	
Marenholz et al, 2006 ³⁵	6.42	2.55	19.63	2.44	1.14	5.45	3.62	2.02	6.82	1.73	1.15	2.59	2.13	1.60	2.83	2.13	1.68	2.71
Palmer et al, 2006 ⁹	9.22	4.17	21.24	15.77	5.37	58.07	11.56	5.73	24.74									
Palmer et al, 2006 ⁹	—	—	—	—	—	—	2.64	1.92	3.63									
Palmer et al, 2006 ⁹	—	—	—	—	—	—	2.27	1.23	4.37									
Ruether et al 2006, ³⁶	3.39	1.75	6.58	NA	NA	NA	NA	NA	NA	2.17	1.33	3.56	—	—	—	—	—	—
Weidinger et al 2006, ^{28†}	4.10	2.20	7.90	2.80	1.60	4.70	3.60	2.30	5.70	3.82*	1.97*	7.42*	2.43*	1.59*	3.72*	2.73*	1.87*	3.98*
Weidinger et al 2007, ³¹	3.59	1.52	9.89	5.07	2.53	11.33	4.33*	2.43*	7.70*									
Barker et al 2007, ^{33‡}	5.09	3.51	7.39	7.27	4.59	11.51	6.29	4.62	8.63									
Betz et al 2007, ³⁷	—	—	—	—	—	—	2.14	1.24	3.66									
Hubiche et al 2007, ^{38†}	3.35	1.39	8.62	9.14	3.10	39.48	5.59	2.81	12.06									
Lerbaek et al 2007, ^{34§}	3.39	0.69	13.16	2.61	0.55	9.49	2.84	0.99	7.47									
Lerbaek et al 2007, ³⁴	2.14	0.45	7.82	2.14	0.45	7.82	2.32	0.77	6.29									
Morar et al 2007, ²⁹	2.55	1.50	4.34	1.93	1.23	3.03	2.03	1.46	2.81	2.55	1.68	3.86	2.38	1.54	3.67	1.72	1.32	2.24
Novak et al 2008, ^{39†}	6.25	1.55	25.22	4.86	1.77	13.33	6.78	2.76	16.64									
Rogers et al, 2007 ⁶	3.09	1.41	7.04	2.02	1.09	3.75	2.38	1.48	3.90	1.38	0.55	3.42	3.75	1.24	11.30	2.40	1.15	5.02
Sandilands et al, 2007 ¹²	14.05	8.18	25.09	8.78	4.97	15.99	8.18¶	5.69¶	11.97¶									
Stemmler et al, 2007 ⁴⁰	1.23	0.65	2.27	1.93	1.29	2.89	3.82	2.46	6.09									
Brown et al, 2008 ¹⁵	5.55	3.74	8.34	4.13	2.67	6.40	5.61¶	4.08¶	7.82¶									
Brown et al, 2008 ¹⁴	1.58	0.87	2.78	2.02	1.08	3.69	1.75¶	1.19¶	2.56¶									
Ekelund et al, 2008 ⁴¹										2.68	1.34	5.33	1.85	1.24	2.76	1.81	1.31	2.50
Giardina et al, 2008 ¹⁷	5.96#	0.28#	125.03#	1.78	0.29	13.65	—	—	—									
Henderson et al, 2008 ³²	2.50	1.91	3.27	2.43	1.87	3.14	2.48*	2.06*	2.99*									
Rice et al, 2008 ^{42†}	2.08	1.29	3.36	1.30	0.74	2.26	1.69	1.14	2.49									
Weidinger et al, 2008 ^{13†}	3.66	2.13	6.22	2.78	1.94	3.95	3.05**	2.29**	4.04**									
Weidinger et al, 2008 ³⁰	—	—	—	—	—	—	4.27	3.28	5.56	3.42	1.80	6.50	2.32	1.52	3.54	2.75	1.91	3.95
Weidinger et al, 2008 ³⁰	—	—	—	—	—	—	5.47	3.71	8.08									
Weidinger et al, 2008 ³⁰	—	—	—	—	—	—	2.27	1.90	2.70									
Pooled estimate	3.54	2.62	4.80	2.97	2.31	3.83	3.39	2.73	4.23	2.23	1.80	2.76	2.18	1.82	2.61	2.03	1.77	2.34
Pooled estimate, both study types	3.14	2.45	4.03	2.78	2.28	3.39	3.12	2.57	3.79									

CC, Case-control studies; FAM, family studies; NA, not available.

*Not considered for meta-analysis because the cohort was included in Weidinger et al.³⁰

†Recalculated from original data.

‡Not considered for meta-analysis because the cohort was included in Brown et al.¹⁵§Comparison with Copenhagen Prospective Study on Asthma in Childhood control subjects was not considered for meta-analysis because the control subjects were used in Palmer et al.⁹

||The case-control approach was not included for pooled estimates, both study types, because cases and control subjects resemble family study offspring.

¶Combined genotype for mutations R501X, 2282del4, R2447X, S3247X, and 3702delG.

#No carriers of R501X were detected in the control group, and ORs were calculated with 2 × 2 contingency tables according to the method of Walter et al.¹⁸

**Combined genotype for mutations R501X, 2282del4, R2447X and S3247X.

RESULTS

A total of 89 publications, including published abstracts (35 from PubMed and 54 from the ISI Web of Knowledge) were identified, of which 24 met the inclusion criteria for the current meta-analyses on eczema, asthma, or both. Tables I* and II† list all case-control and family-based studies included and show the estimated ORs and 95% CIs for both R501X and 2282del4 and the combined genotype, if provided.

The 2 family panels in Morar et al²⁹ were combined into 1 panel, and the 2 individual panels are not displayed separately.

The family cohort in Weidinger et al²⁸ was enlarged with additional families and reanalyzed in Weidinger et al.³⁰ Therefore the former was excluded from the meta-analysis to prevent duplication. The same applies to the case-control study in Weidinger

et al,³¹ which was reanalyzed in Weidinger et al³⁰ for the combined genotype.

For the study by Sandilands et al,¹² the Irish case series analyzed in the original report on *FLG* and atopic eczema by Palmer et al⁹ was enlarged with an additional 136 cases. Because the control populations in both studies have very different sample sizes ($n = 186$ vs 736), we considered both studies independent and assessed their influence in a sensitivity analysis. The same case series enlarged by an additional 230 cases was included in the study by Weidinger et al,³⁰ but the sample size of the control cohort differs largely, and thus these cohorts were also considered independent.

The Avon Longitudinal Study of Parents and Children was analyzed twice for the effect of *FLG* mutations on eczema risk,^{30,32} and hence the study by Henderson et al³² was not considered in the meta-analysis of the combined genotype and eczema.

The case cohort used by Barker et al³³ was extended and reanalyzed by Brown et al,¹⁵ and therefore the former was dropped from the meta-analysis.

*See references 6, 9, 12–15, 17, and 28–42.

†See references 6, 9, 13, 14, 29, 31, 35, 37, 38, and 40–43.

TABLE II. Association studies of *FLG* polymorphisms and asthma that met the inclusion criteria

Study	Asthma			Asthma with eczema			Asthma without eczema		
	OR	95% CI		OR	95% CI		OR	95% CI	
Marenholz et al, 2006 ^{35*}	3.55	1.87	7.02	5.84	2.69	13.12	2.47	1.01	5.71
Marenholz et al, 2006 ^{35†}				2.79	1.65	4.71			
Palmer et al, 2006 ⁹				12.94	5.05	35.93			
Palmer et al, 2006 ⁹	1.73	1.31	2.30	2.64	1.92	3.63	0.97	0.64	1.44
Palmer et al, 2006 ⁹				2.32	0.75	6.27			
Weidinger et al, 2006 ²⁸				3.40	1.68	6.88			
Betz et al, 2007 ³⁷				4.95	2.29	10.36			
Hubiche et al, 2007 ^{38‡}				5.73	1.88	16.78			
Morar et al, 2007 ²⁹	1.52	1.13	2.05				1.28	0.61	2.48
Rogers et al, 2007 ⁶	1.52	0.93	2.47	2.40	1.15	5.02	1.00	0.51	1.96
Weidinger et al, 2007 ^{31§}				6.26	3.12	12.57			
Basu et al, 2008 ^{43‡}	1.61	1.08	2.40						
Brown et al, 2008 ^{14,**}	1.23	0.82	1.81	2.16	1.34	3.40			
Ekelund et al, 2008 ⁴¹				3.58	1.99	6.42			
Henderson et al, 2008 ³²	1.28	0.99	1.63	3.38	2.36	4.74	0.78	0.42	1.32
Rice et al, 2008 ⁴²	1.19	0.90	1.57						
Weidinger et al, 2008 ^{13§,***}	1.79	1.22	2.63	3.49	2.00	6.08	1.45	0.83	2.56
Pooled estimate	1.48	1.32	1.66	3.29	2.84	3.82	1.11	0.88	1.41

*Multicenter Allergy Study cohort.

†Genetic Studies in Nuclear Families with Atopic Dermatitis cohort.

‡ORs for the dominant model.

§Recalculated from original data.

**Combined genotype for mutations R501X, 2282del4, R2447X, S3247X, and 3702delG.

***Combined genotype for mutations R501X, 2282del4, R2447X, and S3247X.

It appears that the studies by Palmer et al⁹ and Lerbaek et al³⁴ used the same control subjects from the Copenhagen Prospective Study on Asthma in Childhood cohort. Because Lerbaek et al³⁴ also compared the cases derived from the Danish Twin Register with unrelated healthy control subjects from the same register, we included this setting in the meta-analyses.

Eczema

The initial aim of this study was to provide a general and overall measure of effect size based on all available association studies on *FLG* and eczema. However, because the study characteristics varied widely (eg, in terms of definitions of eczema, study size and power, source of control subjects, case-control ratio, and phenotyping procedures), we also carried out a sensitivity analysis by grouping the studies based on their characteristics and a meta-analysis that involved only the most robust studies according to generally accepted guidelines (see Table E1 in this article's Online Repository at www.jacionline.org).^{44,45}

Case-control studies

A total of 24 case-control studies on *FLG* and eczema were identified and provided data on the *FLG* combined genotype. Twenty of these studies also provided data for R501X, and 19 provided data for 2282del4, respectively. For the combined genotype, we estimated an overall allelic risk of 3.39 (95% CI, 2.73-4.23) by applying a random-effects model. ORs in the individual studies ranged from 1.69 to 11.56 (Table I and Fig 1), reflecting the large heterogeneity of 83.3%. There was no significant deviation from the symmetry assumption ($P = .2247$), and thus with additional visual inspection, there is no hint of publication bias (Fig 1). For R501X, an OR of 3.54

(95% CI, 2.62-4.80) was calculated in a random-effects model, whereas the OR for 2282del4 was 2.97 (95% CI, 2.31-3.83). For both mutations, no evidence of publication bias was detected by means of formal testing when the symmetry assumption held ($P = .422$ and $P = .194$, respectively; Fig 1).

Family studies

The 6 family studies showed a more homogeneous range of ORs (ie, from 1.72 to 2.75 for the combined genotype; Table I). This suggests a fixed-effects model because the heterogeneity measure shows 0% for both polymorphisms and 20.5% for the combined genotype. The overall OR was estimated 2.03 (95% CI, 1.77-2.34) for the combined genotype, 2.23 (95% CI, 1.80-2.76) for R501X, and 2.18 (95% CI, 1.82-2.61) for 2282del4. No significant deviation from the symmetry assumption could be detected, indicating no publication bias.

Combined analysis of all studies

Because of the interdependence, the case-control study from Morar et al²⁹ was not included. When combining family-based and case-control studies, we did not detect statistically significant asymmetry for the combined genotype ($P = .1316$), R501X ($P = .371$), and 2282del4 ($P = .078$), although by trend, family studies are more homogeneous and show lower ORs than case-control studies (Fig 1). In a random effects-model we estimated an overall OR of 3.12 (95% CI, 2.57-3.79) for the combined genotype, 3.14 (95% CI, 2.45-4.03) for R501X, and 2.78 (95% CI, 2.28-3.39) for 2282del4, respectively. Studies demonstrated considerable variation in frequency of mutations, in particular in cases and offspring, with carrier frequencies ranging from 0.144 to 0.558 (see Table E2 in this article's Online Repository at

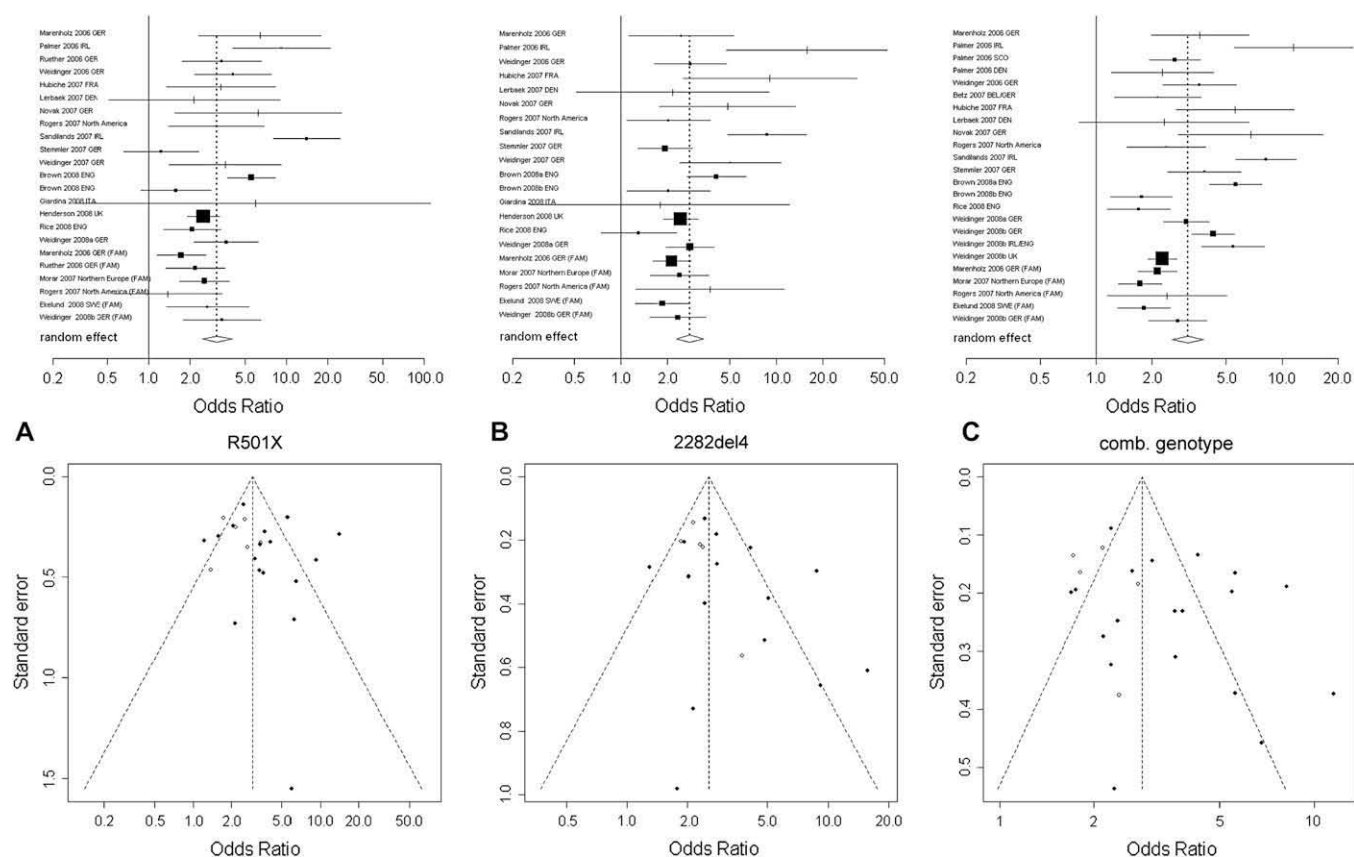


FIG 1. Association of *FLG* mutations R501X and 2282del4 and the combined genotype with increased eczema risk. Forest plots show the ORs and respective 95% CIs for the different studies included in the meta-analysis. Funnel plots show the ORs and SEs for the association. **A**, R501X. **B**, 2282del4. **C**, Combined genotype. Family studies are indicated by *FAM* in the forest plots and by *open circles* in the funnel plots.

www.jacionline.org). Based on the information given in the publications, cases and control subjects were reasonably similar in ancestry. None of the studies adjusted for population stratification (eg, by using non-disease markers).

Grouped analysis based on study characteristics

To account for differences in terms of study design, we performed a sensitivity analysis by grouping the studies based on their characteristics as far as reported in the publications or by means of direct correspondence with the investigators. For all groups, we calculated an overall estimate and restricted analysis to the combined genotype. The studies used in the sensitivity analysis and the grouping criteria are outlined in Table III.‡

Of the 25 studies included in this meta-analysis, 4 were population-based studies (overall OR for eczema, 2.19), 5 were family-based studies (OR, 2.04), and 16 were case-control studies. Five of the 16 case-control studies used population-based control subjects (OR, 4.92), whereas 7 studies used healthy outpatients as control subjects (OR, 2.77), and 4 studies involved other control subjects, such as asthmatic patients without eczema (OR, 5.09). The definition and diagnosis of eczema also differed

between the studies. In 12 studies eczema was diagnosed by dermatologists by using validated criteria, such as the UK Working Party criteria or those of Hanifin and Rajka. The overall OR for eczema from these studies was 4.24 (95% CI, 3.09-5.81). In 5 studies eczema was physician diagnosed (overall OR, 2.07; 95% CI, 1.73-2.48), whereas in 4 studies cases were ascertained on the basis of a reported physician's diagnosis in the past (overall OR, 2.33; 95% CI, 1.83-2.97), and in 4 studies other criteria were applied (overall OR, 2.46; 95% CI, 1.97-3.10). Subanalysis of studies on the basis of case/control subject ratio showed overall estimates of 3.52 (<1:4), 3.25 (1:4-1:2), and 3.48 (>1:2), respectively. According to the number of cases examined, we estimated overall ORs of 2.79 (95% CI, 2.02-3.84) for studies involving more than 500 cases, 2.49 (95% CI, 1.92-3.23) for studies involving 200 to 500 cases, and 3.87 (95% CI, 2.70-5.54) for studies involving less than 200 cases. Finally, studies were grouped according to the severity of eczema in cases based on information given in the publications or provided by the investigators through direct correspondence. Studies on cases with predominantly moderate-to-severe eczema yielded a significantly higher OR of 5.16 (95% CI, 3.92-6.80) in contrast to ORs of 2.70 (95% CI, 2.01-3.64) and 2.11 (95% CI, 1.88-2.38) for studies on predominantly mild-to-moderate eczema cases and studies on inhomogeneous cases or cases with unknown severity, respectively. All results are presented in Fig 2.

‡See references 6, 9, 12-15, 28-30, 35, and 37-42.

TABLE III. Association studies of the *FLG* combined genotype and eczema used in the sensitivity analysis

Study	OR	95% CI	Design	Diagnosis	No. of cases	No. of control subjects	Total no.	Case-control ratio	Eczema severity
Marenholz et al, 2006 ³⁵	3.62	2.02 6.82	3	2	189	321	510	>0.5	B
Palmer et al, 2006 ⁹	11.56	5.73 24.74	4	1	52	186	238	0.25-0.5	A
Palmer et al, 2006 ⁹	2.64	1.92 3.63	2	4	279	1008	1287	0.25-0.5	C
Palmer et al, 2006 ⁹	2.27	1.23 4.37	3	2	142	190	332	>0.5	C
Weidinger et al, 2006 ²⁸	3.60	2.30 5.70	3	1	90	862	952	<0.25	C
Betz et al, 2007 ³⁷	2.14	1.24 3.66	4	4	145	473	618	0.25-0.5	C
Hubiche et al, 2007 ³⁸	5.59	2.81 12.06	4	1	99	102	201	>0.5	A
Lerbaek et al, 2007 ³⁴	2.32	0.77 6.29	3	3	26	157	183	<0.25	C
Morar et al, 2007 ²⁹	2.03	1.46 2.81	3	2	657	333	990	>0.5	C
Rogers et al, 2007 ⁶	2.38	1.48 3.90	3	4	185	461	646	0.25-0.5	C
Sandilands et al, 2007 ¹²	8.18	5.69 11.97	2	1	188	736	924	0.25-0.5	A
Stemmler et al, 2007 ⁴⁰	3.82	2.46 6.09	3	1	378	700	1078	>0.5	A
Brown et al, 2008 ¹⁵	5.61	4.08 7.82	2	1	186	1035	1221	<0.25	A
Brown et al, 2008 ¹⁴	1.75	1.19 2.56	1	1	195	610	805	0.25-0.5	B
Novak et al, 2007 ³⁹	6.78	2.76 16.64	2	1	56	1446	1502	<0.25	B
Rice et al, 2008 ⁴²	1.69	1.14 2.49	1	3	267	5022	5289	<0.25	C
Weidinger et al, 2008 ¹³	3.05	2.29 4.04	1	3	540	2559	3099	<0.25	B
Weidinger et al, 2008 ³⁰	4.27	3.28 5.56	2	1	773	3992	4765	<0.25	A
Weidinger et al, 2008 ³⁰	5.47	3.71 8.08	4	1	418	552	970	>0.5	A
Weidinger et al, 2008 ³⁰	2.27	1.90 2.70	1	3	1583	6063	7646	0.25-0.5	B
Marenholz et al, 2006 ³⁵	2.13	1.68 2.71	0	2	490		490		C
Morar et al, 2007 ²⁹	1.72	1.32 2.24	0	2	426		426		C
Rogers et al, 2007 ⁶	2.40	1.15 5.02	0	4	NA		NA		C
Ekelund et al, 2008 ⁴¹	1.81	1.31 2.50	0	1	406		406		C
Weidinger et al, 2008 ³⁰	2.75	1.91 3.95	0	1	486		486		A

Design: 0, family-based study; 1, population-based (cross-sectional) study; 2, case-control study with population control subjects; 3, case-control study with outpatient control subjects with known eczema status; 4, other. Diagnosis: 1, dermatologist's diagnosis according to validated criteria; 2, physician's diagnosis according to validated criteria; 3, patient-reported physician's diagnosis; 4, other. Eczema severity: A, predominantly moderate to severe; B, predominantly mild to moderate; C, not reported or inhomogeneous. NA, Not available.

In addition, we carried out a meta-analysis on the most robust studies only according to generally accepted standard criteria (ie, after exclusion of studies that appeared to violate predefined criteria, such as Hardy-Weinberg equilibrium).^{44,45}

Asthma

To further clarify the inconclusive association between *FLG* and asthma, we conducted a meta-analysis of 9 studies that analyzed the trait asthma, 6 studies that analyzed the trait asthma without eczema, and 14 studies that analyzed asthma plus eczema. For all subphenotypes, we calculated an overall estimate and restricted the analyses to the combined *FLG* genotype. Studies showed considerable differences concerning the definition of asthma (eg, physician's diagnosis, self-report, or both) that were not always specified in detail.

For asthma analyzed independently from eczema status, we estimated an overall OR of 1.48 (95% CI, 1.32-1.66) by using a fixed-effects model because of an observed heterogeneity of 40.9% ($P = .095$). The test of asymmetry did not indicate any publication bias ($P = .066$). However, visually, the funnel plot shows some weak asymmetry caused by the study by Marenholz et al,³⁵ which might have overestimated the effect.

A stronger effect was observed for the subphenotype asthma plus eczema, with an overall OR of 3.29 (95% CI, 2.84-3.82) in a fixed-effects model (heterogeneity of 39.5%, $P = .064$). There does not appear to be an obvious publication bias toward positive findings, but both the funnel plot and the Egger test ($P = .039$) indicated that the effect for asthma plus eczema might have been

overestimated in the small-sized Irish cohort within the initial study by Palmer et al.⁹

In contrast, 5 of 6 somewhat homogeneous studies found no significant association, with asthma without eczema, yielding an overall OR estimate of 1.11 (95% CI, 0.88-1.41) by applying a fixed-effects model (test of heterogeneity, $P = .284$). There was no indication toward publication bias when the symmetry assumption held ($P = .193$). All results are presented in Table II and Fig 3.

DISCUSSION

This meta-analysis of genetic association studies on the most common *FLG* variants in European populations involved 5,791 eczema cases and 26,454 control subjects, as well as 1,951 families. In addition, 3,138 asthma cases, 17,164 control subjects, and 4 family studies with 1,511 asthma-affected offspring were analyzed. This analysis provides the most comprehensive assessment thus far of the relevance of common *FLG* polymorphisms to eczema and represents the first meta-analysis on their effect on asthma. In a previous meta-analysis that involved less than half the number of individuals of the present study, we found a relative risk for eczema of about 3.58 under a dominant genetic model. The same model needs to be fitted to each dataset to combine case-control and family studies into one analysis. It is possible to fit a dominant model to family data by using conditional logistic regression,^{20,46} but this would require individual-level family genotype data, which are not available. Thus to enhance direct comparability between case-control and family-based studies, we used regression methods to estimate the multiplicative allelic

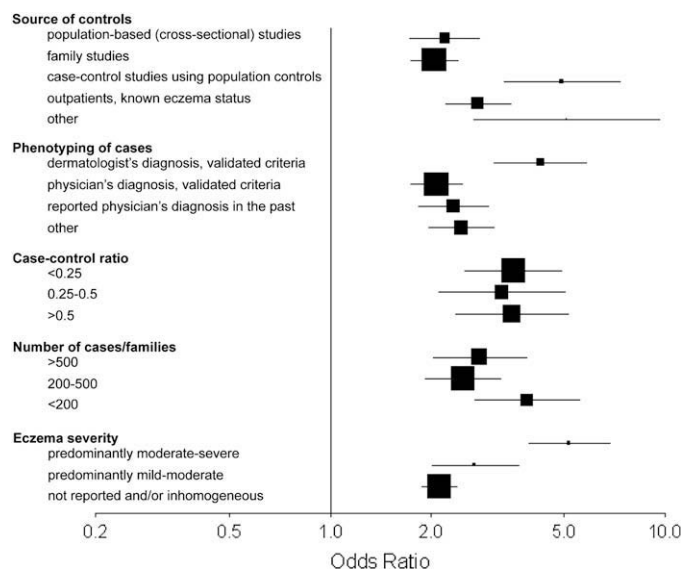


FIG 2. Association of *FLG* polymorphisms (combined genotype) with increased eczema risk grouped by study characteristics.

relative risk in both case-control and family studies.²¹ However, results do not differ significantly from those obtained through a dominant model of analysis (combining the heterozygote with the homozygote mutants to compare carriers of ≥ 1 *FLG* null mutations with wild-type individuals, see Tables E3 and E6).

Our current analysis demonstrates that across all studies there is a high risk conferred by both null polymorphisms, with an estimated overall OR of 3.14, 2.78, and 3.12 for R501X, 2282del4, and the combined genotype, respectively, without formal evidence of publication bias. However, it has to be considered that visual inspection of funnel plots is not completely reliable and that statistical tests that are used to quantify the asymmetry have various limitations.⁴⁷ Furthermore, there appear to be large differences in allele frequencies across Europe, depending on the countries in which the participants live and their ethnicity. It has to be noted that although R501X and 2282del4 are common in populations of European ancestry, they are absent in Asian populations, in which a different spectrum of *FLG* variants has been identified. To date, nothing is known about the role of *FLG* mutations in other ethnic groups. Because none of the studies published thus far have adjusted for population stratification, inflated false-positive rates cannot be completely ruled out. However, based on the information given in the publications and provided by the authors through correspondence, cases and control subjects of the single studies did not differ significantly with respect to their ethnic background. In addition, results from case-control studies are strongly supported by family-based studies, which are robust to stratification, and do not differ significantly from those obtained from the most robust case-control studies (see Table E1 in this article's Online Repository at www.jacionline.org). Thus it appears unlikely that population stratification had a significant influence on the outcomes. The OR for eczema is higher for R501X than for 2282del4 in this meta-analysis, suggesting a possible differential risk effect with mutation site; however, there are insufficient large studies on the other uncommon recurrent null alleles to explore this possibility further. Interestingly, the effect of *FLG* on eczema in population-based cross-sectional studies, in which we assume no population stratification, is nearly the same as in family studies,

further strengthening our findings and conclusions to the general population (Fig 2, Table I, and see Table E4 in this article's Online Repository at www.jacionline.org).

Although environmental factors are undoubtedly important for the development of eczema in susceptible individuals, it has been estimated in twin studies that almost 80% of the predisposition to eczema is due to genetic factors.⁴⁸⁻⁵¹ Eczema and atopic diseases do not follow a simple Mendelian mode of inheritance but represent complex traits with several genes that might be relevant. Thus far, *FLG* has shown the strongest effect, and the results from this meta-analysis clearly illustrate that the magnitude of its effect on the risk of eczema is higher than that of any other confirmed candidate gene for atopic diseases and one of the largest ever shown in the genetics of complex diseases.

Although an impressive number of studies in various cohorts have demonstrated a strong effect of *FLG* variants on eczema risk, with only 2 negative reports (in studies with methodological weaknesses), our overall results showed considerable between-study heterogeneity. This could be due to bias, chance, or real differences in populations or in interactions with other risk factors. Thus attention should be given to the characteristics of the individual studies. Although eczema cases were apparently selected according to appropriate criteria, it has to be considered that eczema is quite a heterogeneous disease, with considerable variability in age of onset, presentation, severity, distribution, and course. Furthermore, there were large differences in terms of study size and power and control selection. Studies also differed in the extent of testing to exclude control subjects with eczema, and in many of them, screening and ascertainment of control subjects were not described in detail. Moreover, none of the studies performed to date adjusted for population stratification.

However, the considerably extended data available at the time of this study allowed better refinement of *FLG* risk profiles within the broad and inclusive diagnosis of eczema. Interestingly, we detected a clear signal that the strongest associations for *FLG* within eczema are in dermatologist-diagnosed cases and severe-to-moderate cases (OR of 4.24 [95% CI, 3.09-5.81] and OR of 5.16 [95% CI, 3.92-6.80], respectively). Because of incomplete data in many of the

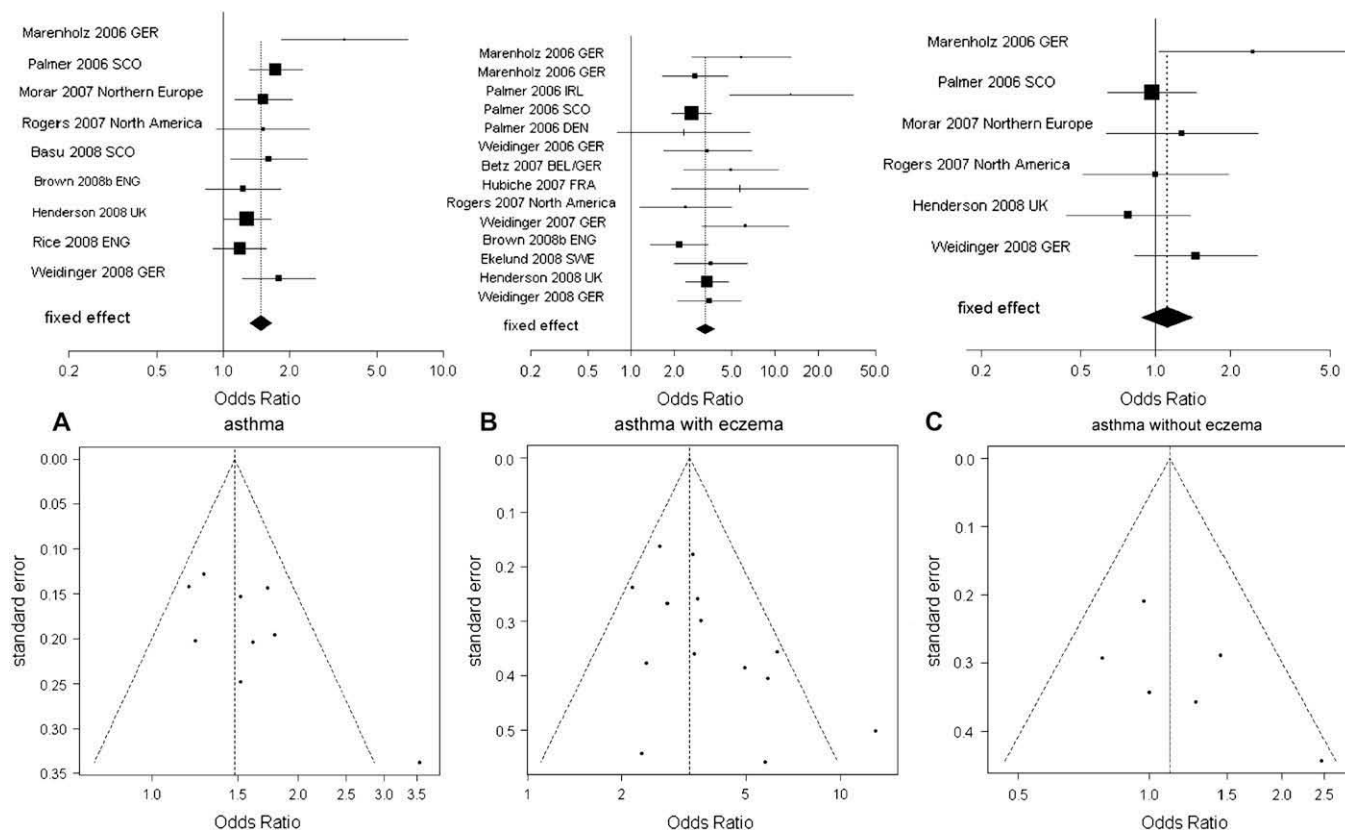


FIG 3. Association of *FLG* polymorphisms (combined genotype) with increased risk to several asthma definitions: **A**, Asthma. **B**, Asthma with eczema. **C**, Asthma without eczema. Forest plots show the ORs and respective 95% CIs for the different studies included in the meta-analysis. Funnel plots show the ORs and SEs for the association.

published studies, we were unable to properly analyze differential effects on atopic versus nonatopic eczema, as has been suggested previously.^{13,28,30,31,52} Although the data are not sufficiently robust to ascertain cases as atopic versus nonatopic eczema in all cohorts, in those in which such a clear differentiation was possible, a differential effect is seen, with a stronger OR for atopic eczema (2.86) versus nonatopic eczema (2.47; see Table E5 in this article's Online Repository at www.jacionline.org).

Whereas the association of *FLG* alleles with more severe and dermatologist-diagnosed disease appears real and robust, the mechanisms that underlie this observation remain unclear. One possibility is that in addition to being a susceptibility factor for initiation of eczema, within established eczema, *FLG* null alleles function as a severity factor and amplify disease. Although speculative, this is consistent with previous longitudinal population data that suggest a persistence effect on eczema duration conferred by *FLG* null alleles.³² More severe, more persistent, or both types of disease might also have an effect on asthma development.

Although the strong effect of a genetically determined *FLG* deficiency on eczema risk is undoubted and consistent with its expression profile and putative functions, a role for *FLG* in asthma has been hitherto less clear. Several studies have suggested that *FLG* mutations also confer an increased risk for the development of asthma. However, there was a large degree of heterogeneity between these studies (eg, in definition of asthma and sample sizes)

that might have confounded the analysis. In addition, most of the studies investigated subjects with asthma ascertained through a diagnosis of eczema, whereas only a few examined asthma independently from eczema status, either in population-based cohorts or cohorts ascertained on the basis of asthma (Table II). Meta-analysis of the latter studies showed that *FLG* null alleles are significantly associated with asthma *sensu lato*, with a notable OR of 1.5. This compares quite strongly with other asthma susceptibility genes.^{53,54} In particular, the association with asthma and eczema is strongest for the complex phenotype of eczema plus asthma (OR, 3.29). Further analysis based on the available data indicates that the association with asthma appears to be limited to asthma with coexpression of eczema, whereas *FLG* mutations appear to not independently affect the risk for asthma. This observation is supported by results from recent large-scale and robust studies.^{6,13,32} Thus it seems conceivable that *FLG* deficiency might predispose to the particular asthma phenotype occurring in the context of eczema in contrast to the form of asthma not linked with eczema and that eczema is on the mechanistic pathway to conferral of risk.

The mechanism of the asthma risk associated with *FLG* null alleles is not yet fully understood. *FLG* is expressed in the skin⁹ and in the outer layers of the oral and nasal mucosae^{13,55} but not in the respiratory epithelium of the nose or the lower airways.^{13,56} Therefore it has been suggested that *FLG*-associated asthma is

mediated through percutaneous priming⁵⁷ and/or secondary, possibly systemic, immunologic mechanisms stimulated through the impaired skin barrier. Interestingly, recent large-scale studies have shown that the risk of asthma (and rhinitis) is not restricted to those with allergic sensitization,^{13,32} suggesting that the mechanistic association of eczema and later asthma cannot be simplistically reduced to excess allergen sensitization and IgE production caused by epithelial barrier failure. Clearly, further epidemiologic and mechanistic studies are needed to explore the link between *FLG* deficiency and airway disease.

In conclusion, we report here a comprehensive analysis encompassing all qualifying studies on *FLG* mutations and eczema and asthma published to January 2009. *FLG* null alleles are seen to confer a high risk of eczema, with a greater effect on moderate-to-severe disease and dermatologist-diagnosed disease and a trend toward a stronger effect with atopic or extrinsic disease. Additionally, the overall risk of asthma conferred by *FLG* null alleles is shown to be approximately 1.5 but importantly only in asthma in the context of eczema, suggesting that the complex eczema plus asthma phenotype is a distinct endophenotype of both asthma and eczema. These data suggest that *FLG*-related eczema might have a distinct phenotype and disease trajectory within the current broad definitions of eczema.

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Key messages

- The effect of *FLG* on eczema risk is higher than that of any other confirmed candidate gene for atopic diseases and one of the largest ever shown in the genetics of complex diseases.
- The strongest associations for *FLG* within eczema are in dermatologist-diagnosed cases and moderate-to-severe cases.
- *FLG* deficiency predisposes to the particular asthma phenotype occurring in the context of eczema in contrast to the form of asthma not linked with eczema.

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METHODS

In addition to the allelic inheritance model, a dominant inheritance model was applied for case-control studies, and results of both models were compared. In Table E3 all case-control studies for *FLG* and eczema are displayed, whereas in Table E6 all case-control studies on *FLG* and asthma are shown.

If available, allele and carrier frequencies for case-control studies were calculated, and pooling was done according to the method proposed by Thakkinian et al.^{E1} Furthermore, where appropriate, Hardy-Weinberg equilibrium for each study's control group was assessed by use of the χ^2 test.

The most robust association studies according to generally accepted criteria^{E2,E3} were the subject of a separate meta-analysis, which was carried out as described in the Methods section. The following studies were included: Marenholz et al.^{E4} (case-control and family study), Brown et al.^{E5} Weidinger et al.^{E6} Weidinger et al.^{E7} (all 3 case-control studies and the family study), Ekelund et al.^{E8} and Morar et al.^{E9} (family study). Because of interdependence with 1 or more of these studies, the following studies had to be excluded: Weidinger et al.^{E10} Morar et al.^{E9} (case-control study), and Henderson et al.^{E11}

For the population-based (cross-sectional) studies, the population-attributable risk (PAR) for eczema was calculated as follows: $PAR = [PrevE (OR - 1)] / [1 + PrevE (OR - 1)]$, where *PrevE* is the pooled prevalence of exposure (proportion of *FLG* mutation carriers in the population), as determined by using the method of Thakkinian et al.^{E1} and *OR* is the pooled OR from the meta-analysis.^{E12}

RESULTS

For eczema, the overall estimates obtained by using a dominant inheritance model did not differ significantly from those obtained by using the allelic inheritance model, whereas for asthma, the effects were slightly different. The lower bound of the 95% CI is higher in the dominant models compared with that in the allelic models. In summary, both inheritance models show consistent results (Table E3, Table E6, and Table I).

Meta-analyses on the most robust association studies on *FLG* and eczema yielded an overall OR of 2.96 (95% CI, 2.29–3.82; Table E1). Meta-analyses of the population-based studies showed a pooled OR of 2.19 (95% CI, 1.72–2.80), with a pooled carrier frequency (combined genotype) of 0.093 (95% CI, 0.051–0.135) and an overall PAR of 10.0% (Table E4).

For atopic eczema, higher ORs were observed compared with those for eczema in general; however, the CIs overlap, and the estimates for atopic eczema are within the CI for eczema (Table E5).

There are only 2 studies that provide information on atopic asthma. For Henderson et al.,^{E11} we calculated an OR of 2.26 (95% CI, 1.32–3.65), and for Weidinger et al.,^{E6} an OR of 1.78 (95% CI, 0.99–3.18) was observed. Although for Henderson et al.^{E11} this estimate is higher than that for asthma, for Weidinger et al.,^{E6} it is nearly identical.

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TABLE E1. Most robust association studies of *FLG* polymorphisms and eczema

Study	CC									FAM								
	R501X			2282del4			Combined			R501X			2282del4			Combined		
	OR	95% CI		OR	95% CI		OR	95% CI		OR	95% CI		OR	95% CI		OR	95% CI	
Marenholz et al, 2006 ^{E4}	6.42	2.55	19.63	2.44	1.14	5.45	3.62	2.02	6.82	1.73	1.15	2.59	2.13	1.60	2.83	2.13	1.68	2.71
Weidinger et al, 2007 ^{E10}	3.59	1.52	9.89	5.07	2.53	11.33	4.33*	2.43	7.70									
Morar et al, 2007 ^{E9†}	2.55	1.50	4.34	1.93	1.23	3.03	2.03	1.46	2.81	2.55	1.68	3.86	2.38	1.54	3.67	1.72	1.32	2.24
Brown et al, 2008 ^{E5}	5.55	3.74	8.34	4.13	2.67	6.40	5.61‡	4.08	7.82									
Ekelund et al, 2008 ^{E8}										2.68	1.34	5.33	1.85	1.24	2.76	1.81	1.31	2.50
Henderson et al, 2008 ^{E11}	2.50	1.91	3.27	2.43	1.87	3.14	2.48*	2.06	2.99									
Weidinger et al, 2008 ^{E6}	3.66	2.13	6.22	2.78	1.94	3.95	3.05§	2.29	4.04									
Weidinger et al, 2008 ^{E7}	—	—	—	—	—	—	4.27	3.28	5.56	3.42	1.80	6.50	2.32	1.52	3.54	2.75	1.91	3.95
Weidinger et al, 2008 ^{E7}	—	—	—	—	—	—	5.47	3.71	8.08									
Weidinger et al, 2008 ^{E7}	—	—	—	—	—	—	2.27	1.90	2.70									
Pooled estimate + 95% CI	3.57	2.52	5.05	2.81	2.20	3.60	3.48	2.54	4.76	2.32	1.81	2.97	2.14	1.78	2.58	2.02	1.75	2.33
Pooled estimate, both study types	3.10	2.35	4.10	2.54	2.14	3.00	2.96	2.29	3.82									

CC, Case-control studies; FAM, family studies.

*Not considered for meta-analysis because the cohort was included in Weidinger et al.⁷

†This case-control study was not included in the pooled estimate of both study types because the cohort was included in the family study.

‡Combined genotype for mutations R501X, 2282del4, R447X, S3247X, and 3702delG.

§Combined genotype for mutations R501X, 2282del4, R2447X, and S3247X.

TABLE E2. Allele frequencies, Hardy-Weinberg equilibrium, carrier frequencies, and ethnic origin of subjects in case-control studies for *FLG* polymorphisms and atopic eczema

Study	Allele frequencies		HWE control subjects	Carrier frequencies		Ethnicity cases/control subjects
	Cases	Control subjects	P value	Cases	Control subjects	
Marenholz et al, 2006 ^{E4}	0.094	0.025	0.643	0.167	0.051	Ger
Palmer et al, 2006 ^{E14}	0.337	0.043	0.540	0.558	0.086	Ire/Ire
Palmer et al, 2006 ^{E14}	0.127	0.049	0.083	0.229	0.093	Sco/Sco
Palmer et al, 2006 ^{E14}	0.095	0.042	0.233	0.176	0.079	Den
Ruether et al, 2006 ^{E15}	NA	NA	NA	NA	NA	Ger/Ger
Weidinger et al, 2006 ^{E16}				0.367	0.138	Ger/Ger
Weidinger et al, 2007 ^{E10*}	0.119	0.030	0.624	0.211	0.06	Ger/Ger
Barker et al, 2007 ^{E27†}	0.273	0.046	0.248	0.423	0.088	GB/GB
Betz et al, 2007 ^{E17}	0.083	0.040	0.376	0.152	0.08	Ger, Net/central Eur‡
Hubiche et al, 2007 ^{E18}	0.177	0.035	0.647	0.313	0.071	Fra/Fra
Lerbaek et al, 2007 ^{E19§}	0.115	0.042	0.235	0.231	0.079	Den/Den
Lerbaek et al, 2007 ^{E19}	0.115	0.057	0.446	0.231	0.115	Den
Morar et al, 2007 ^{E9}	NA	NA	NA	0.267	0.141	Mixed (white subjects, Asian subjects)
Novak et al, 2007 ^{E20}	0.172	0.043	0.745	0.344	0.085	Ger/Ger
Rogers et al, 2007 ^{E21}	0.092	0.035	0.442	0.144	0.071	North American (white non-Hispanic subjects)
Sandilands et al, 2007 ^{E22}	0.287	0.039	0.918	0.452	0.076	Ire/Ire
Stemmler et al, 2007 ^{E23}	0.087	0.022	0.207	0.158	0.043	Ger/Ger
Brown et al, 2008 ^{E5}	0.299	0.059	0.696	0.457	0.115	Eng/Eng
Brown et al, 2008 ^{E24}	0.113	0.065	0.302	0.184	0.129	Eng
Giardina et al, 2008 ^{E25}	NA	NA	NA	NA	NA	Ita/Ita
Henderson et al, 2008 ^{E11*}	0.076	0.032	0.040	0.148	0.064	Eng
Rice et al, 2008 ^{E13}	NA	NA	NA	NA	NA	Eng
Weidinger et al, 2008 ^{E6¶}	0.083	0.029	0.981	0.163	0.058	Ger
Weidinger et al, 2008 ^{E7}	0.123	0.037	0.920	0.221	0.073	Ger/Ger
Weidinger et al, 2008 ^{E7}	0.254	0.049	0.227	0.418	0.098	Eng, Ire/Ire
Weidinger et al, 2008 ^{E7}	0.076	0.036	0.097	0.147	0.072	Eng
Pooled estimate	0.113 (0.079-0.146)	0.037 (0.022-0.052)	—	0.216 (0.174-0.259)	0.075 (0.054-0.096)	

Hardy-Weinberg equilibrium *P* values were calculated for control subjects.

HWE, Hardy-Weinberg equilibrium; Ger, Germany; Ire, Ireland; Sco, Scotland; Den, Denmark; GB, Great Britain; Net, Netherlands; Eur, Europe; Fra, France; Eng, England; Ita, Italy; NA, Not available.

*Not considered for pooling because the cohort was included in Weidinger et al.^{E7}

†Not considered for pooling because the cohort was included in Brown et al.^{E5}

‡No details available.

§Comparison with Copenhagen Prospective Study on Asthma in Childhood control subjects was not considered for pooling because control subjects were used in Palmer et al.^{E14}

||Combined genotype for mutations R501X, 2282del4, R447X, S3247X, and 3702delG.

¶Combined genotype for mutations R501X, 2282del4, R2447X, and S3247X.

TABLE E3. Case-control studies on *FLG* polymorphisms and eczema that met the inclusion criteria by using a dominant genetic model

Study	R501X			2282del4			Combined		
	OR	95% CI		OR	95% CI		OR	95% CI	
Marenholz et al, 2006 ^{E4}	6.65	2.43	18.22	2.42	1.09	5.38	3.73	1.98	7.02
Palmer et al, 2006 ^{E14}	9.22	4.11	20.70	16.76	5.23	53.74	13.40	6.33	28.36
Palmer et al, 2006 ^{E14}	—	—	—	—	—	—	2.89	2.04	4.11
Palmer et al, 2006 ^{E14}	—	—	—	—	—	—	2.49	1.26	4.93
Ruether et al, 2006 ^{E15}	3.59	1.80	7.05	7.10†	3.41†	14.78†	NA	NA	NA
Weidinger et al, 2006 ^{E16}	4.10	2.20	7.90	2.50	1.40	4.30	3.30	2.10	5.40
Weidinger et al, 2007 ^{E10}	3.59	1.43	9.01	5.07	2.42	10.64	—	—	—
Betz et al, 2007 ^{E17}	—	—	—	—	—	—	2.05	1.16	3.62
Hubiche et al, 2007 ^{E18*}	3.35	1.39	8.62	9.14	3.10	39.48	5.59	2.81	12.06
Lerbaek et al, 2007 ^{E19}	2.14	0.54	8.51	2.14	0.54	8.51	2.32	0.82	6.53
Morar et al, 2007 ^{E9*}	2.55	1.50	4.34	1.93	1.23	3.03	2.03	1.46	2.81
Novak et al, 2007 ^{E20†}	6.25	1.55	25.22	4.86	1.77	13.33	6.78	2.76	16.64
Rogers et al, 2007 ^{E21}	3.10	1.36	7.07	1.85	0.93	3.68	2.20	1.26	3.84
Sandilands et al, 2007 ^{E22}	14.05	8.04	24.53	8.94	4.99	16.01	10.02	6.74	14.89
Stemmler et al, 2007 ^{E23}	1.31	0.65	2.62	1.93	1.25	2.96	4.18	2.60	6.73
Brown et al, 2008 ^{E5**}	6.19	4.03	9.51	4.15	2.62	6.56	6.46	4.47	9.32
Brown et al, 2008 ^{E24**}	1.51	0.83	2.75	2.02	1.09	3.72	1.53	0.99	2.37
Giardina et al, 2008 ^{E25}	5.96	0.28	125.04	1.78	0.29	10.79	—	—	—
Henderson et al, 2008 ^{E11}	2.50	1.91	3.27	2.42	1.86	3.14	—	—	—
Rice et al, 2008 ^{E13*}	2.08	1.29	3.36	1.30	0.74	2.26	1.69	1.14	2.49
Weidinger et al, 2008 ^{E6***}	3.66	2.15	6.23	2.86	2.00	4.09	3.16	2.36	4.22
Weidinger et al, 2008 ^{E7}	—	—	—	—	—	—	4.56	3.44	6.05
Weidinger et al, 2008 ^{E7}	—	—	—	—	—	—	5.88	3.85	8.99
Weidinger et al, 2008 ^{E7}	—	—	—	—	—	—	2.23	1.87	2.67
Pooled estimate + 95% CI	3.93	2.73	5.66	3.18	2.41	4.19	3.75	2.87	4.90
Pooled estimate + 95% CI, allelic model	3.84	2.68	5.51	3.18	2.44	4.14	3.59	2.84	4.54

NA, Not available.

*Estimates are calculated with an allelic model by using logistic regression.

†Not used for comparison of the dominant and allelic inheritance models because the allelic model was not applicable.

**Combined genotype for mutations R501X, 2282del4, R2447X, S3247X, and 3702delG.

***Combined genotype for mutations R501X, 2282del4, R2447X, and S3247X.

TABLE E4. Association studies of *FLG* polymorphisms and eczema in population-based (cross-sectional) cohorts

Study	Combined genotype			PAR	Carrier frequencies
	OR	95% CI			
Brown et al, 2008 ^{E24}	1.75*	1.19	2.56	9.6%	0.142
Henderson et al, 2008 ^{E11} †	2.48	2.06	2.99	11.4%	0.087
Rice et al, 2008 ^{E13} ‡	1.69	1.14	2.49		
Weidinger et al, 2008 ^{E6}	3.05§	2.29	4.04	13.6%	0.077
Weidinger et al, 2008 ^{E7}	2.27	1.90	2.70	12.1%	0.087
Pooled estimate	2.19	1.72	2.80	10.0%	0.093

*Combined genotype for mutations R501X, 2282del4, R447X, S3247X, and 3702delG.

†Not included in the meta-analysis because it was included in Weidinger et al.^{E7}

‡Not included in meta-analysis because the carrier frequency is not given.

§Combined genotype for mutations R501X, 2282del4, R2447X, and S3247X.

TABLE E5. Association studies of *FLG* polymorphisms and atopic eczema

Study	R501X			2282del4			Combined		
	OR	95% CI		OR	95% CI		OR	95% CI	
Marenholz et al, 2006 ^{E4}							3.75	1.98	7.40
Morar et al, 2007 ^{E9}							2.28	1.46	3.56
Rogers et al, 2007 ^{E21}							2.40	1.47	3.94
Weidinger et al, 2007 ^{E10}	4.02	1.58	10.26	5.00	2.34	10.69	4.02	1.58	10.26
Henderson et al, 2008 ^{E11}							3.19	2.25	4.45
Weidinger et al, 2008 ^{E6**}							4.56	3.08	6.74
Marenholz et al, 2006 ^{E4} (FAM)	2.09	1.26	3.48	2.38	1.70	3.33	2.49	1.86	3.34
Weidinger et al, 2006 ^{E16} (FAM)	5.71	2.56	12.76	2.30	1.46	3.61	2.93	1.94	4.44
Ekelund et al, 2008 ^{E8} (FAM)	4.33	1.88	9.94	2.22	1.41	3.47	2.21	1.50	3.25
Pooled estimate + 95% CI, atopic eczema	3.18	2.23	4.53	2.47	1.98	3.08	2.86	2.49	3.28
Pooled estimate + 95% CI, eczema	2.47	1.69	3.60	2.32	1.74	3.09	2.47	2.14	2.86

FAM, Family study.

**Combined genotype for mutations R501X, 2282del4, R2447X, and S3247X.

TABLE E6. Case-control studies of *FLG* polymorphisms and asthma that met the inclusion criteria by using a dominant genetic model

Study	Allelic model									Dominant model								
	Asthma			Asthma with eczema			Asthma without eczema			Asthma			Asthma with eczema			Asthma without eczema		
	OR	95% CI		OR	95% CI		OR	95% CI		OR	95% CI		OR	95% CI		OR	95% CI	
Marenholz et al, 2006 ^{E4}	3.55	1.87	7.02	5.84	2.69	13.12	2.47	1.01	5.71	3.61	1.79	4.85	2.22	10.62	14.89	2.47	1.05	5.81
Palmer et al, 2006 ^{E14}				12.94	5.05	35.93							14.17	5.19	38.7			
Palmer et al, 2006 ^{E14}	1.73	1.31	2.30	2.64	1.92	3.63	0.97	0.64	1.44	1.81	1.34	2.46	2.89	2.04	4.11	1.03	0.67	1.57
Palmer et al, 2006 ^{E14}				2.32	0.75	6.27							2.78	0.92	8.42			
Betz et al, 2007 ^{E17}				4.95	2.29	10.36												
Hubiche et al, 2007 ^{E18*}				5.73	1.88	16.78							5.73	1.88	16.78			
Morar et al, 2007 ^{E9}	1.27*	1.02*	1.58*				1.28	0.61	2.48	1.27*	1.02*	1.58*				1.48	0.69	3.14
Weidinger et al, 2007 ^{E10}				6.26	3.12	12.57							6.62	3.25	13.47			
Basu et al, 2008 ^{E26*}	1.61	1.08	2.40							1.61	1.08	2.40						
Brown et al, 2008 ^{E24**}	1.23	0.82	1.81	2.16	1.34	3.40				1.13	0.72	1.77	1.97	1.13	3.45			
Henderson et al, 2008 ^{E11}	1.28	0.99	1.63	3.38	2.36	4.74	0.78	0.42	1.32	1.56	1.17	2.09	3.47	2.42	4.96	0.78	0.44	1.39
Rice et al, 2008 ^{E13*}	1.19	0.90	1.57							1.19	0.90	1.57						
Weidinger et al, 2008 ^{E6***}	1.79	1.22	2.63	3.49	2.10	5.80	1.45	0.83	2.56	1.82	1.22	2.71	3.64	2.11	6.26	1.50	0.84	2.67
Pooled estimate + 95% CI	1.63	1.25	2.12	3.75	2.79	5.04	1.13	0.88	1.46	1.68	1.42	1.99	3.93	2.89	5.36	1.19	0.92	1.55

*Not used for comparison of the dominant and allelic inheritance models because neither the allelic model nor the dominant model was applicable.

**Combined genotype for mutations R501X, 2282del4, R2447X, S3247X, and 3702delG.

***Combined genotype for mutations R501X, 2282del4, R2447X, and S3247X.