Toward a major risk factor for atopic eczema: Meta-analysis of filaggrin polymorphism data

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Background: With an impressive series of replication studies, filaggrin *(FLG)* has become the gene with the most widely replicated association to atopic eczema (AE). However, studies published to date demonstrate differences concerning study design and strength of associations.

Objectives: We sought to provide a general and overall estimate of *FLG* effect sizes and to estimate allele and carrier frequencies.

Methods: We searched Medline and Institute for Scientific Information Web of Knowledge databases for relevant studies and abstracts from professional societies that were published through June 30, 2007. Initially, we accounted for different study types and evaluated an overall estimate for case-control and family studies. In a second step, we combined those 2 study types and used a random-effects analysis approach to calculate overall odds ratios (ORs). Tests of asymmetry were applied to detect potential publication bias.

Results: Nine studies that met the inclusion criteria were included in the meta-analysis. For the combined genotype (R501X or 2282del4), we found an overall OR of 4.09 (95% CI, 2.64-6.33) from the case-control studies and a summary OR of 2.06 (95% CI, 1.76-2.42) from the family studies.

Conclusion: The powerful effect of *FLG* variation on AE risk exceeds that of any other investigated candidate gene for AE thus far and makes *FLG* one of the strongest genes known to date for complex diseases.

Clinical implications: These results underline the importance of a genetically determined epidermal barrier disruption in AE.

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Atopic eczema (AE) is one of the most common inflammatory skin disorders and affects up to 20% of children and up to 10% of adults in developed countries.[1] It is firmly established that AE is under strong genetic control.[2] Its frequent occurrence with other atopic phenotypes, including asthma and allergic rhinitis, suggests shared genetic risk variants.[3] In addition, it is highly probable that there are organ- and disease-specific factors, as indicated by genome-wide scans.[2]

Despite considerable efforts that resulted in numerous proposed or refuted associations with AE susceptibility genes, until recently, no strong and reproducible genetic determinant could be identified. The detection of filaggrin *(FLG)* as major gene for AE in 2006 was an important breakthrough.[4,5] Subsequent studies have shown *FLG* to be the gene with the most widely replicated association to AE.[6-13]

FLG is located within the epidermal differentiation complex (EDC) in a region on chromosome 1q21, with a significant linkage signal to AE and psoriasis.[14,15] The EDC is a cluster of genes and gene families encoding proteins involved in terminal differentiation of the epidermis.[16] The main function of *FLG* is to aggregate keratin filaments, leading to keratinocyte compaction and formation of the stratum corneum.[16,17] It is thereby crucial for engineering and maintaining the barrier function of the skin.

Two *FLG* polymorphisms, R501X and 2282del4, have been shown to exert minor allele frequencies of greater than 0.01 in the white European population.[4,10]

Both variants are in trans and biochemically equivalent, leading to a complete loss of *FLG* expression.[10] They have been shown to cause ichthyosis vulgaris, a common inherited skin disorder of keratinization that is frequently associated and shows many overlapping characteristics with AE.[5,10,18] Subsequently, a variety of case-control and transmission studies firmly established an association between each of the 2 prevalent *FLG* null alleles and AE.[4,6-13] In the meantime, additional less common *FLG* variants associated with AE also in non-European populations have been reported.[10,19]

Thus far, *FLG* is unambiguously the only gene robustly associated with AE, as pointed out by several overview

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Abbreviations used
AE: Atopic eczema
EDC: Epidermal differentiation complex
FLG: Filaggrin
HWE: Hardy-Weinberg equilibrium
OR: Odds ratio

articles.[20-23] However, association studies published thus far show striking differences concerning study design and strength of associations. To gain a better understanding of the contribution of genetic variation in *FLG* to risk of AE and to provide a more accurate estimate of effect sizes, we performed a meta-analysis that combines data and attempts to pool the different study types. Furthermore, we estimated allele and carrier frequencies in control groups.

METHODS

Data extraction

A computerized search of Medline (National Library of Medicine, Bethesda, Maryland, 1966–2007) and the ISI Web of Knowledge–Web of Science (April 2006–April 2007) was conducted by 3 independent researchers (HB, BB, and SW) to identify published studies and abstracts on AE and *FLG* polymorphisms R501X and 2282del4 from the first reported association[4] up to now. We included all articles presenting original data of family or case-control studies on *FLG* and AE. The reference list of the retrieved articles was also reviewed to identify publications on the same topic.

For estimating allele and carrier frequencies in control groups, we included all studies on *FLG* and AE.

In the meta-analysis we included all articles presenting a 3×2 contingency table of *FLG* polymorphisms or combined genotype versus AE and all publications providing odds ratios (ORs) plus 95% CIs. We focused on the association with AE because only few comparable studies are available thus far for related traits.

All studies taken into account presented estimated ORs uncorrected for potential confounders or gave complete contingency tables of *FLG* polymorphisms versus AE. Therefore we used unadjusted estimates to quantify the OR of *FLG* carriers versus noncarriers. Adjusted ORs were only available for a small subset of studies considered here.

We excluded all articles without data for the effect of the genetic variations on AE risk.

Statistical analysis

Because the 2 common *FLG* mutations R501X and 2282del4 are in trans and have equivalent biologic effects,[10] association analysis was also performed for a combined genotype in most of the studies. In other words, individuals carrying one mutated and one wild-type allele were coded heterozygous, and individuals heterozygous for each of the 2 polymorphisms or homozygous for 1 polymorphism were coded homozygous for the combined genotype. Because we were interested in the overall magnitude of the *FLG* effect, we focused our analysis on this combined genotype.

In our initial approach, we accounted for different study types and evaluated an overall estimate for case-control and family studies separately. Considering transmission of possible alleles A and B from parents to an affected offspring, in a trio setting the ratio of transmitted A and untransmitted B alleles (T) to untransmitted A and transmitted B alleles (U) can be interpreted as an OR according to Morris and Gardner,[24] as follows:

$$OR = \frac{T}{U}.$$

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

ORs in case-control studies were calculated for *FLG* carriers versus noncarriers if contingency tables were available. A symmetric 95% CI was calculated for log(OR) by using the normal approximation of the Pearson-Mantel-Haenzel statistic.[25]

In a second step we combined those 2 study types and estimated an overall OR.

We estimated the overall effect of *FLG* null alleles with a random-effects model, as described by DerSimonian and Laird.[26] The test of homogeneity was performed according to the method of Cooper and Hedges.[27] To detect a potential publication bias, funnel plots were constructed, and the test for asymmetry by Egger et al[28] was applied.

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 AE imparted by *FLG* variations for individual studies, group analyses, and combined analyses of all groups. Funnel plots might provide a hint on publication bias.

Additionally, the frequency of the mutant alleles in R501X and 2282del4 was estimated in control subjects or parents by using the inverse variance method, as described in Thakkinstian et al.[29] Furthermore, where appropriate, we assessed Hardy-Weinberg equilibrium (HWE) for each study's control group or parents by use of the χ^2 test. Finally, where available, we estimated the prevalence of polymorphisms among cases. For all analyses, we used the base-, stats- and meta-packages in R 2.4.1.[30,31]

RESULTS

Our literature search yielded 39 publications, of which 9 met the inclusion criteria for the meta-analysis. Table I[4,6-13] shows the study type, the population origin, and the number of cases and control subjects or nuclear families. In addition, reported ORs and their corresponding 95% CIs are given. We did not identify abstracts with original data to be included in the analysis.

Morar et al[8] analyzed 2 different family panels and presented results for each panel separately, as well as for the combined panels. In a sensitivity analysis we compared the results of our meta-analyses under consideration of the results achieved in this combined panel and the results for the separate panels. Because no significant differences could be detected (Table I) for the overall estimate, both panels were subsequently considered as one cohort. In addition, Morar et al[8] investigated the risk of disease in affected offspring and unaffected children from the family panels in a case-control setting. Because these study settings are not independent, this case-control approach was not used for the meta-analyses across study types. The study on an Irish case-control cohort by Palmer et al[4] was included in a more comprehensive analysis in

TABLE I. Unadjusted ORs and 95% CIs of studies on the association of *FLG* variants with AE included in the meta-analysis

Study	Type	Population	n	R501X		2282del4		Combined genotype		Diagnosis of AE based on:
				OR	95% CI	OR	95% CI	OR	95% CI	
Weidinger et al[12]	Fam	Germany	476	3.82	2.02-7.68	2.43	1.60-3.76	2.73	1.89-4.01	UK Working Party
Weidinger et al[13]	cc	Germany	274/252	3.59	1.43-9.01	5.07	2.42-10.64	4.17	2.30-7.59	UK Working Party
Marenholz et al[7]	Fam	Europe	490*	1.73	1.16-2.61	2.13	1.61-2.84	2.13	1.68-2.72	Hanifin and Rajka
	cc	Germany	188/319	6.65	2.43-18.22	2.42	1.09-5.38	3.73	1.98-7.03	Hanifin and Rajka
Barker et al[6]	cc	United Kingdom	163/1463	5.85	3.86-8.86	7.61	4.74-12.21	7.59	5.30-10.87	UK Working Party
Palmer et al[4]	cc	Denmark	142/190†	—	—	—	—	2.49†	1.26-4.93†	Hanifin and Rajka
	cc	Scotland	279/1008‡	—	—	—	—	2.89‡	2.04-4.11‡	Questionnaire
Ruether et al[9]	Fam	Germany	338	2.17	1.34-3.61	—	—	—	—	Hanifin and Rajka
	cc	Germany	272/276	3.59	1.80-7.05	7.1	3.41-14.78	—	—	Hanifin and Rajka
Morar et al[8]	cc§	Northern Europe#	426/564	2.55	1.50-4.34	1.93	1.23-3.03	2.03	1.46-2.81	—
	Fam§	Northern Europe#	426	2.55	1.70-3.90	2.38	1.55-3.71	1.72	1.32-2.25	Modified Hanifin and Rajka
	Fam‖	Northern Europe#	148	2.40	1.15-5.02	3.00	1.60-5.62	1.94	1.27-2.95	—
	Fam¶	United Kingdom	278	2.62	1.58-4.33	1.88	1.02-3.44	1.58	1.12-2.24	—
Stemmler et al[11]	cc	Germany	378/700	1.31	0.65-2.62	1.93	1.25-2.96	4.18	2.60-6.73	Hanifin and Rajka
Sandilands et al[10]	cc	Ireland**	188/736	14.05	8.04-24.53	8.94	4.99-16.01	10.02‡‡	6.74-14.89	UK Working Party
Pooled estimate + 95% CI			cc	4.23	2.36-7.59	4.13	2.38-7.16	4.09	2.64-6.33	—
			Fam	2.32	1.73-3.09	2.25	1.83-2.77	2.10	1.66-2.65	—
			Total††	3.51	2.24-5.50	3.62	2.43-5.41	3.58	2.43-5.26	—
Continental Europeans			Total	2.61	1.81-3.78	2.82	1.99-4.00	2.97	2.28-3.87	—

Fam, Family study; *cc*, case-control study; *Total*, combined analysis of both study types.
*Four hundred ninety families including 903 children with eczema.
†Children of mothers with asthma.
‡Patients with asthma and eczema.
§Consists of individuals belonging to nuclear families of 2 panels.
‖ECZ1 panel of northern Europe (90% white, 1% Asian, and 4.3% mixed + others).
¶MRCE panel (69% white, 14% South Asian, and 8% mixed + others).
#Inclusive United Kingdom.
**Irish study by Palmer et al[4] is included in Sandilands et al.[10]
††Morar (case-control) excluded.
‡‡OR includes 3 additional rare variants.

Sandilands et al,[10] which we therefore used for the present analysis.

All studies included were conducted on western European populations, in particular from Germany, Great Britain, and Ireland.

For pooled analysis of carrier frequencies, cases and control groups from 17 case-control studies and parents and offspring from 5 family studies were used. The study by Stemmler et al[11] was excluded from this analysis because of a distortion of HWE for R501X (Table II).[4-13,32-34] In addition, the Irish AE case series within the study by Palmer et al[4] was excluded because these cases have later been included into the Sandilands study.[10] Nine other studies were not considered because of incomplete or missing data. Among the control subjects, no significant heterogeneity was observed. Allele frequencies were estimated at 1.9% (95% CI, 0.3%-3.5%) for R501X, 1.9% (95% CI, 0.5%-3.3%) for 2282del4, and 4.1% (95% CI, 1.4%-6.8%) for the combined genotype (Table II).[4-13,32-34] In contrast, striking differences were observed concerning the carrier frequencies in cases between Ireland/United Kingdom ranging from 22.9% to 45.2% and continental Europe ranging from 15.8% to 22.9%. Because of low sample sizes in Ireland/United Kingdom cohorts, the differences remained nonsignificant (Table III).[4-13,32-34]

Case-control studies

To date, 9 independent case-control studies on *FLG* and AE have been performed across western Europe, 8 of which provided data on both polymorphisms, showing an increased risk for AE ranging from 2.03 to 10.02 for the combined allele (Table I and Fig 1).

After applying a random-effects model, an overall OR was estimated at 4.09 (95% CI, 2.64-6.33, $P = 9.2 \times 10^{-10}$). Observed heterogeneity between the studies was 87.1% (95% CI, 76.7%-92.8%). The test of asymmetry showed no significant deviation from the symmetry assumption ($P = .973$), thus not indicating any publication bias (Fig 2).

For both null alleles, a strong overall *FLG* effect was calculated with an OR of 4.23 (95% CI, 2.36-7.59; range, 1.3-14.1) for R501X and 4.13 (95% CI, 2.38-7.16; range, 1.9-8.9) for 2282del4, respectively. Tests of asymmetry were not significant ($P = .6237$ and $P = .3988$), indicating no publication bias.

After excluding the study of Stemmler et al[11] because of distortion of HWE concerning the R501X allele, applying a random-effects model, we then estimated an overall OR

TABLE II. Minor allele frequencies of *FLG* variants (R501X, 2282del4, and combined genotype) and HWE test results observed in studies on *FLG* published until April 2007

			R501X			2282del4			Combined genotype		
					HWE			HWE			HWE
Study			n*	MAF	*P* value	n*	MAF	*P* value	n*	MAF	*P* value
Weidinger et al[12]	Fam	Germany	930	2.8%	.371	952	5.5%	.238	930	8.2%	.319
Weidinger et al[13]	cc	Germany	251	1.2%	.848	250	1.8%	.772	249	3.0%	.624
Marenholz et al[7]†	Fam	Europe	970	2.8%	NS	958	6.6%	NS	–	9.3%	NS
	cc	Germany	319	0.8%	.888	316	1.7%	.753	314	2.5%	.643
Barker et al[6]	cc	United Kingdom	1463	2.9%	.102	1463	1.7%	.515	1463	4.6%	.248
Palmer et al[4]	cc	Ireland**	189	3.2%	.652	186	1.1%	.882	186	4.3%	.540
	cc	Denmark	–	–	–	–	–	–	190	4.2%	.233
	cc	Scotland	1008‡	3.0%	.026	1008	1.9%	.542	1008‡	4.9%	.831
	pop§	France	158	2.5%	–	155	1.0%	–	–	–	–
	pop§	Ireland/Scotland	242	2.8%	–	257	1.0%	–	–	–	–
Ruether et al[9]	Fam‡	Germany	–	–	–	–	–	–	–	–	–
	Cc‡	Germany	–	2.2%	–	–	1.7%	–	–	–	–
Morar et al[8]	cc	Northern Europe#	–	–	NS	–	–	NS	–	–	NS
	Fam	Northern Europe#	–	–	NS	–	–	NS	–	–	NS
	Fam	United Kingdom	–	–	NS	–	–	NS	–	–	NS
Stemmler et al[11]	cc	Germany	700‡	1.6%	8.0×10^{-6}	678	3.5%	.831	630c	2.2%	.207
Sandilands et al[10]	cc	Ireland	736	1.3%	.723	736	1.3%	.723	736	3.9%	.918
Zhao et al[32]§	cc	Ireland	654	1.5%	.691	654	1.6%	.676	654	3.1%	.408
	cc	United Kingdom	1463	2.9%	.102	1463	1.7%	.515	1463	4.6%	.248
Hüffmeier et al[33]§‖	cc	Germany	369	1.8%	NS	360	2.8%	NS	–	–	–
Smith et al[5]§	pop	Ireland	–	4.1%	–	–	0.5%	–	–	–	–
	pop	Scotland	–	2.1%	–	–	1.2%	–	–	–	–
	pop	United States¶	124	2.4%	–	133	1.1%	–	–	–	–
Gruber et al[34]§	pop	Austria	–	1.4%	–	–	1.4%	–	–	2.7%	–
Pooled allele frequency (95% CI)			1.9% (0.3% to 3.5%)			1.9% (0.5% to 3.3%)			4.1% (1.4% to 6.8%)		
Continental European			1.7% (0% to 4.0%)			2.8% (0.2% to 5.3%)			4.1% (0% to 8.5%)		

MAF, Minor allele frequency; *Fam*, family study; *cc*, case-control study; *NS*, not significant; –, information not available; *pop*, population-based cohort.
*Number of control subjects in case-control–based studies, number of parents in family-based studies, and total number in population-based studies.
†Calculated with stated genotyping rate of 0.99 and 0.977 for R501X and 2282del4, respectively.
‡Not pooled because of violation of HWE or missing number of successfully genotyped control subjects.
§Control subjects from studies on related phenotypes.
‖Calculated with stated genotyping rate of 0.981 and 0.957 for R501X and 2282del4, respectively.
¶European Americans.
#Inclusive United Kingdom.
**Not pooled because of inclusion in Sandilands et al.[10]

of 5.16 (95% CI, 3.02-8.81) and 4.13 (95% CI, 2.38-7.16) for R501X and the combined genotype, respectively. Tests of asymmetry remained not significant, indicating no publication bias.

Family studies

The 5 family studies evaluated showed ORs from 1.58 to 2.73 for the combined genotype. These rather homogeneous estimates result in an overall OR of 2.10 (95% CI, 1.66-2.65; $P = 2.0 \times 10^{-9}$) by applying a random-effects model (Fig 1). A fixed-effects model revealed similar results (OR, 2.06; 95% CI, 1.76-2.42). The heterogeneity test revealed no significant deviation from the homogeneity assumption ($P = .1364$). Further investigation of R501X and 2282del4 revealed an overall OR of 2.32 (95% CI, 1.73-3.09) and 2.25 (95% CI, 1.83-2.77) by applying a random-effects model. Heterogeneity measures showed no significance ($P = .2198$ and $P = .8435$, respectively).

Combined analysis of all studies

Combining family-based and case-control studies, the test of asymmetry remains not significant ($P = .1994$, Fig 2), although ORs in family studies tend to be lower with smaller CIs than in case-control studies. Because of the interdependence, the case-control studies from Morar et al[8] were not included. We also excluded the Irish case-control study in Palmer et al[4] because the respective individuals were included in a subsequent study by Sandilands et al.[10]

The estimated overall OR for a random-effects model was 3.51 (95% CI, 2.24-5.50) for R501X, 3.62 (95% CI, 2.43-5.41) for 2282del4, and 3.58 (95% CI, 2.43-5.26; $P = 3.7 \times 10^{-10}$) for the combined genotype.

Having further investigated Table I and Fig 1, the OR estimates appeared lower for continental European populations. Therefore we calculated a pooled estimate of studies conducted in continental Europe only. Applying a random-effects model, we observed an estimate of 2.61 (95% CI, 1.81-3.78) for R501X, 2.82 (95%

TABLE III. Carrier frequencies of *FLG* variants (R501X, 2282del4, and combined genotype) in cases and control subjects for case-control studies or offspring and parents for family studies in studies on *FLG* published until April 2007

			R501X		2282del4		Combined genotype	
			Carrier frequency		Carrier frequency		Carrier frequency	
Study			Control subjects/ parents	Cases/ offspring	Control subjects/ parents	Cases/ offspring	Control subjects/ parents	Cases/ offspring
Weidinger et al[12]	Fam	Germany	5.7%	8.8%	10.9%	15.8%	16.0%	22.8%
Weidinger et al[13]	cc	Germany	2.4%	8.1%	3.6%	15.9%	6.0%	21.1%
Marenholz et al[7]	Fam	Europe	–	–	–	–	–	22.9%
	cc	Germany	1.6%	9.6%	3.5%	8.0%	5.1%	16.7%
Barker et al[6]	cc	United Kingdom	5.6%	25.8%	3.4%	20.9%	8.8%	42.3%
Palmer et al[4]	cc	Ireland‖	6.4%	38.5%	2.2%	26.9%	8.6%	55.8%
	cc	Denmark	–	–	–	–	7.9%	17.6%
	cc	Scotland	5.8%*	–	3.8%	–	9.3%*	22.9%
	pop†	France	–	–	–	–	–	–
	pop†	Ireland/Scotland	–	–	–	–	–	–
Ruether et al[9]	Fam	Germany	–	–	–	–	–	–
	cc	Germany	–	–	–	–	–	–
Morar et al[8]	cc	Northern Europe§	–	–	–	–	–	–
	Fam	Northern Europe§	–	–	–	–	–	–
	Fam	United Kingdom	–	–	–	–	–	–
Stemmler et al[11]	cc	Germany	2.9%*	3.7%	6.8%	12.3%	4.3%*	15.8%
Sandilands et al[10]	cc	Ireland	2.6%	27.1%	2.6%	19.1%	7.6%	45.2%
Zhao et al[32]†	cc	Ireland	3.1%	n.AE	3.2%	n.AE	6.3%	n.AE
	cc	United Kingdom	5.6%	n.AE	3.4%	n.AE	8.8%	n.AE
Hüffmeier et al[33]†	cc	Germany	–	n.AE	–	n.AE	–	n.AE
Smith et al[5]†	pop	Ireland	–	n.AE	–	n.AE	–	n.AE
	pop	Scotland	–	n.AE	–	n.AE	–	n.AE
	pop	United States‡	–	n.AE	–	n.AE	–	n.AE
Gruber et al[34]†	pop	Austria	–	n.AE	–	n.AE	–	n.AE
Pooled allele frequency (95% CI)			3.5% (0.9% to 6.0%)	9.0% (3.1% to 15.0%)	3.8% (1.5% to 6.2%)	14.7% (8.0% to 21.4%)	8.0% (4.3% to 11.7%)	22.9% (16.4% to 29.3%)
Continental European			2.8% (0% to 6.8%)	6.4% (0.7% to 12.1%)	5.7% (0.9% to 10.4%)	12.6% (5.0% to 20.3%)	8.2% (0.2% to 14.2%)	22.9% (15.1% to 30.7%)

Fam, family study; *cc*, case-control study; –, information not available; *pop*, population-based cohort; *n.AE*, other trait than AE.
*Not pooled because of distortion of the HWE.
†Control subjects from studies on *FLG* with related phenotypes.
‡European American.
§Inclusive United Kingdom.
‖Not pooled because of inclusion in Sandilands et al.[10]

CI, 1.99-4.00) for 2282del4, and 2.97 (95% CI, 2.28-3.87) for the combined genotype. Between the studies, there was no evidence for significant heterogeneity ($P = .074$).

DISCUSSION

In this study we have conducted the most comprehensive assessment of currently available data on the effect of the 2 most common *FLG* polymorphisms in European populations on AE. Our comprehensive meta-analysis is based on an impressive series of replication studies that have followed the initial publication from Palmer et al[4] in early 2006. Although these studies exhibited a considerable heterogeneity concerning study design and the magnitude of the genetic effect, they have firmly established *FLG* as major gene for AE. Our analysis demonstrates that across all studies there is a high risk independently conferred by both null polymorphisms, with an estimated overall OR of 3.51, 3.62, and 3.58 for R501X, 2282del4, and the combined genotype, respectively. Thus the powerful effect of *FLG* variation on AE risk exceeds that of any other investigated candidate gene for AE thus far.[2] Comparison with effects observed for candidate genes of other complex diseases (eg *ADAM33* and asthma[35] or *TCF7L2* and diabetes[36]) shows that *FLG* is also one of the strongest known genes for complex diseases in general. With values of 57% and 49% for breast cancer by age 75 years, *BRCA1* and *BRCA2* mutations show penetrance rates comparable with *FLG* and AE.[37] However, in contrast to *FLG*, *BRCA* mutations account for less than 5% of all breast cancer cases.[38]

Although frequencies of at least one of the null alleles were comparable among control subjects, strikingly higher carrier frequencies were observed in British and Irish case collections (22.9% to 45.2%) along with a

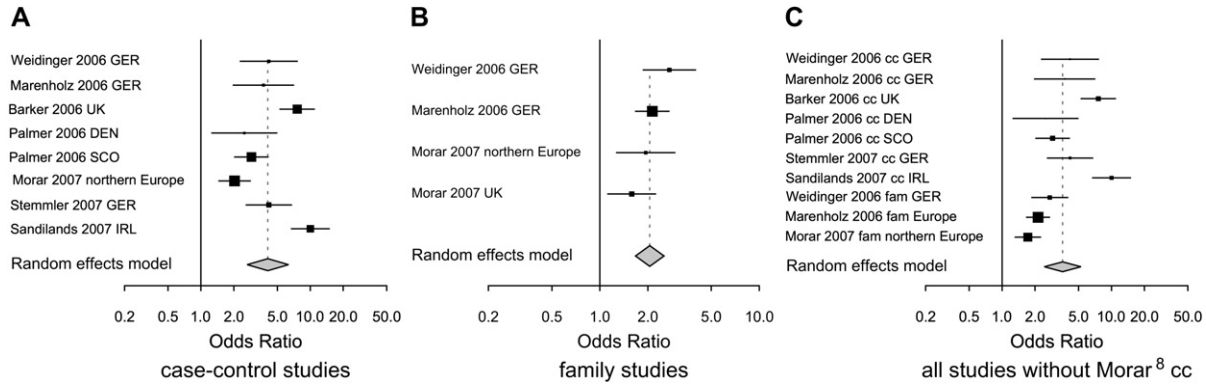

FIG 1. Association of *FLG* mutations R501X and 2282del4 (combined genotype) with increased AE risk. Forest plots showing the ORs and respective 95% CIs for the different studies (given by first author, year, and ethnicity of the study population) included in the meta-analysis are shown: **A**, case-control studies; **B**, family studies; **C**, combined analysis. The rhombus designates the overall estimate of all studies. The consistently higher AE risk conferred by *FLG* null alleles in all studies is reflected by a high overall estimated OR. *GER*, Germany; *UK*, United Kingdom; *DEN*, Denmark; *SCO*, Scotland; *IRL*, Ireland; *cc*, case control study.

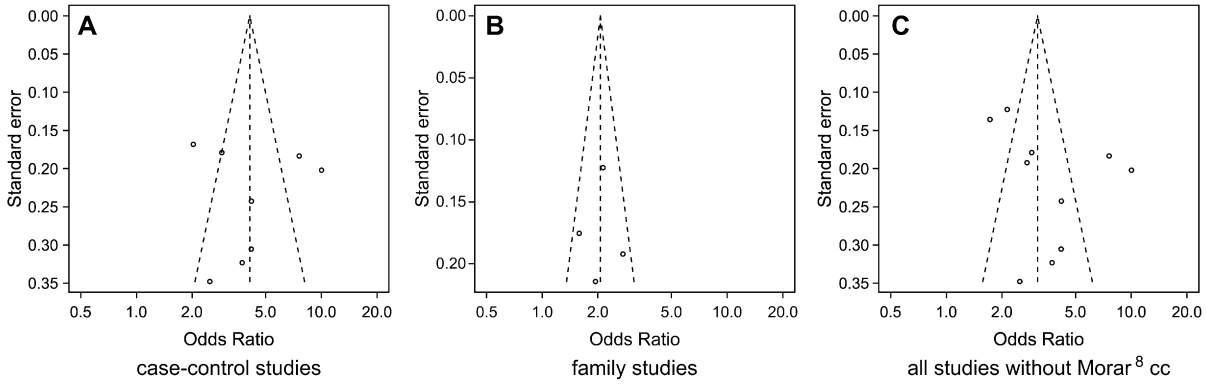

FIG 2. Funnel plots showing the ORs and 95% CIs for the association between *FLG* mutations R501X and 2282del4 (combined genotype) and AE observed in case-control studies **(A)**, family studies **(B)**, and both study types (combined analysis; **C**). Tests of asymmetry showed no significant deviation from the symmetry assumption (Fig 2, *A*, $P = .973$; Fig 2, *B*, $P = .953$; Fig 2, *C*, $P = .199$), thus not indicating publication bias.

higher disease risk (OR, 2.03-10.02) compared with studies from mainland Europe (15.8% to 22.9%). Background minor allele frequencies for R501X are lower in continental Europe, which partially explains this difference. Other possible explanations might include variation in disease spectrum, such as differences in severity and age ranges or differences concerning unknown environmental factors.

Our findings must be taken with caution for a number of reasons. First, these estimates are obtained by pooling despite heterogeneous study designs (eg, case-control and family-based studies). Family-based studies include probands who are designated as having AE as a result of being examined for the purpose of completing the study, but the eczema might not be so severe as to need medical attention. In contrast, case-control studies deal with unrelated individuals who, in the majority of cases, have sufficiently severe AE to require attendance to secondary care. Thus these studies cannot be directly compared. Second, the selection of cases and control subjects varied substantially, and the clinical characteristics of cases and control subjects were often not sufficiently specified. Some groups of control subjects were population based,[4,9,13] whereas others were hospital based.[11] Most studies did not control for known risk factors for AE. In addition, some studies lack information regarding the assessment of Hardy-Weinberg proportion in control subjects, such as the study by Stemmler et al,[11] in which reanalysis showed that R501X appears to be not in HWE.

A rather homogeneous estimate of the *FLG* risk on AE could be observed for the family studies, possibly reflecting the known additional level of control for occult genetic admixture, which might confound case-control studies. However, it has to be considered that the constructed ORs[24] are not directly comparable with those obtained from case-control studies.

We suggest that despite the issues discussed, this large study has established *FLG* polymorphisms as an extraordinarily strong marker of AE disease risk because we found no evidence of bias. We identified and considered

major methodological issues of the various studies, and significant results were observed in all of the studies performed thus far, as well as in the pooled analysis. Given that additional prevalent variants have recently been described,[10] the current estimate of overall contribution of *FLG* to AE is likely an underestimate, with additional recurrent polymorphisms likely to be revealed in specific populations.

Clearly, well-defined and large-scale, population-based and longitudinal studies are needed to more precisely work out the contribution of *FLG* polymorphisms to AE risk, to evaluate their role in other atopic diseases, and to determine their effect in the general population. In addition, because it has been shown that *FLG* polymorphisms are not solely responsible for the significant genetic linkage signal to the EDC region on chromosome 1q21,[8] further investigation of this complex locus is likely to identify additional genetic variants with effects on AE.

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