

Filaggrin mutations confer susceptibility to atopic dermatitis but not to asthma

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Background: Loss-of-function mutations in the filaggrin gene (*FLG*) have been strongly associated with atopic dermatitis and allergic phenotypes in multiple populations. The role of these mutations in the development of asthma is less clear, particularly in patients who do not have coincident atopic dermatitis.

Objective: To determine whether *FLG* mutations are associated with asthma or asthma-related intermediate phenotypes.

Methods: We genotyped 2 loss-of-function *FLG* mutations (R501X and 2282del4) in white children (age 5-12 years) with mild to moderate asthma in the Childhood Asthma Management Program. We assessed the relationship of these mutations to asthma and allergy-related phenotypes in children with and without atopic dermatitis using both population-based and family-based tests of association.

Results: Nearly 1/3 (185/646) of the participating children had atopic dermatitis. Although strong associations were observed between *FLG* mutations and atopic dermatitis (odds ratio, 2.4; $P = 7.6 \times 10^{-5}$) and between the mutations and total serum IgE level ($P = .009$ in the atopic dermatitis cohort), no association was noted with either asthma or asthma-related phenotypes, including FEV₁, FEV₁/forced vital capacity, and methacholine PC₂₀ ($P > .1$ for all tests).

Conclusion: Although *FLG* loss-of-function mutations are consistently associated with atopic dermatitis and other allergic phenotypes, these mutations do not appear to influence either susceptibility to asthma or asthma severity phenotypes.

Clinical implications: Filaggrin mutations that predispose to atopic dermatitis do not modulate the asthma phenotype. (J Allergy Clin Immunol 2007;120:1332-7.)

Key words: Atopic dermatitis, genetics, asthma, filaggrin, association

The filaggrin gene (*FLG*, GenBank accession number NM_002016), located on chromosome 1q21, has recently been identified as an important susceptibility locus for ichthyosis vulgaris and atopic dermatitis (AD).^{1,2} The gene is expressed in epidermal keratinocytes as a polypeptide that undergoes posttranslational cleavage into multiple filaggrin subunits. These molecules aggregate keratin filaments to establish skin barrier function. Two loss-of-function mutations (a nonsense mutation [R501X] and a 4-bp deletion [2282del4] causing frameshift), both resulting in protein truncation, are present in populations of European ancestry at a combined frequency as high as 5% to 10%.^{1,3} First identified in 15 multiplex families with ichthyosis vulgaris,² these mutations confer a semi-dominant phenotype with incomplete penetrance: homozygotes typically manifest ichthyosis vulgaris, whereas heterozygotes exhibit milder dermal abnormalities more reminiscent of AD.

In a follow-up study of these initial observations, Palmer et al¹ demonstrated strong associations of these same mutations with AD in multiple European populations, with odds ratios (ORs) of 3.3 to 13.0 for carriers of the null mutations. These findings have been replicated in numerous populations,³⁻⁸ with OR for AD consistently between 2.8 and 13,⁹ resulting in estimated population attributable risk (PAR) fractions of 11% for AD in white populations.⁵ Although causality is difficult to establish without experimental support, these strong and consistent data clearly establish *FLG* mutations as among the most important risk factors for the development of AD and represent one of the strongest genotype-phenotype relationships reported among complex traits.

The role of filaggrin in mediating asthma risk is less clear. In several of the aforementioned studies,^{1,5} these mutations also demonstrated strong association with other allergic phenotypes, including asthma, with the PAR for asthma among subjects with concomitant AD estimated at 20.6%.⁵ However, these *post hoc* analyses should be interpreted cautiously, given that these subjects were primarily ascertained through a diagnosis of AD; the *FLG* associations with asthma were restricted to subjects who also carried a diagnosis of AD, and not mutation carriers who had asthma in the absence of AD.^{1,3,5,8} Moreover, *FLG* does not

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Abbreviations used

AD: Atopic dermatitis
CAMP: Childhood Asthma Management Program
FLG: Filaggrin
FVC: Forced vital capacity
OR: Odds ratio
PAR: Population attributable risk
SNP: Single nucleotide polymorphism

appear to be expressed in the bronchial mucosa, largely precluding direct phenotypic effects in the lung.¹⁰ To clarify the role of filaggrin as an asthma susceptibility gene, we genotyped both polymorphisms in a family-based cohort ascertained through children with mild-to-moderate asthma participating in the Childhood Management Asthma Program (CAMP) ancillary genetic study. Herein we report that although we are able to confirm the strong association of filaggrin with AD and related atopic phenotypes, these polymorphisms do not appear directly to influence susceptibility to asthma *per se*.

METHODS

Population

The CAMP is a multicentered North American clinical trial designed to investigate the long-term effects of inhaled anti-inflammatory medications in children with mild to moderate asthma.^{11,12} A diagnosis of asthma was based on methacholine hyperreactivity (PC₂₀ no greater than 12.5 mg/mL) and 1 or more of the following criteria for at least 6 months in the year before recruitment: (1) asthma symptoms at least 2 times per week, (2) at least 2 uses per week of an inhaled bronchodilator, or (3) daily asthma medication. Protocols for collection of baseline phenotypic data have been described in detail elsewhere.^{11,12} A diagnosis of AD was based on an affirmative response to the question, "Has your child ever had atopic dermatitis (eczema) for 2 consecutive years"?

Of the 1041 children enrolled in the original clinical trial, 968 children and 1518 of their parents contributed DNA samples. Because of the small number of samples from nonwhite ethnic groups and concerns regarding genetic heterogeneity, we restricted genotyping to the self-described white subjects and their available parents (646 probands and 460 complete nuclear families, including 42 families with multiple affected offspring).

The Institutional Review Boards of the Brigham and Women's Hospital and of the other CAMP study centers approved this study. Informed assent and consent were obtained from the study participants and their parents to collect DNA for genetic studies.

Genotyping

2282del4 (located at UCSC hg18 position chr 1: 150, 551, 701-704) was characterized by PCR-based fragment length polymorphism analysis. R501X (located at UCSC hg18 position chr 1: 150,552,485) was genotyped by using the 5' → 3' exonuclease probe hybridization assay (TaqMan; Applied Biosystems, Foster City, Calif). Primers and probes for both assays were described by Palmer et al.¹ Reactions were optimized by using 48 random DNA samples. Fragment length analysis was determined by using the 3730 DNA

analyzer (Applied Biosystems), and TaqMan fluorescent output was measured with the 7900HT Sequence Detection System (ABI). Approximately 3% of the sample was randomly genotyped at both loci twice to determine genotype reproducibility rates. Genotype quality was determined by assessing genotype completion and reproducibility rates and confirming Mendelian patterns of marker inheritance.

Statistical analysis

Population-based analysis was performed by using SAS v.9.1 (SAS Institute, Cary, NC). Phenotypes tested included AD, log-transformed total serum IgE level, skin test reactivity to at least 1 allergen, log-transformed methacholine PC₂₀, FEV₁, and the ratio of FEV₁ to forced vital capacity (FVC). All binary and categorical variables were assessed by using the Fisher exact test. Continuous variables were assessed by using linear regression, adjusted for age and sex (and height where applicable).

Family-based testing and power calculations were performed by using PBAT v.5.3.0 (C.G Lambert, HelixTree Genetics Analysis Software, Golden Helix, Inc, <http://www.goldenhelix.com>, Bozeman, Mont).^{13,14} Asthma affection status was evaluated, as were all variables assessed in the population-based analysis. Haploview version 3.32 (Broad Institute, Boston, Mass) was used to obtain counts of allele transmissions in family-based analysis.¹⁵

RESULTS

Genotyping

Two loss-of-function *FLG* mutations were genotyped in CAMP participants and their available parents (total N genotyped = 2260). The baseline characteristics of the 646 white CAMP subjects are presented in Table I. Twenty-nine percent (n = 185) had AD. Those with AD were slightly younger, and as expected had higher IgE levels and positive skin prick testing to at least 1 allergen.

Genotype completion rates were 97.8% and 98.5% for 2282del4 and R501X, respectively. No discordances were observed for either locus on repeat genotyping of 81 subjects (3.6% of the total sample), and no inconsistencies in marker inheritance were observed among white probands. Parental genotypic data were in Hardy-Weinberg equilibrium ($P > .1$ for both mutations). Among probands, 8.7% carried at least 1 *FLG* mutation, including 3.7% for R501X and 5.6% for 2282del4. Because both mutations are located proximally in the gene (within the first of 12 filaggrin repeats) and result in premature filaggrin protein truncation, we considered them functionally equivalent in our primary analysis by constructing a combined genotype. One null mutation constitutes a "heterozygote," and 2 mutations (either true homozygotes or compound heterozygotes) constitute a "homozygote." This same technique has been used in multiple previous studies.^{1,3,5}

Of those 611 probands with genotype data at both loci, 90.8% had no mutations (homozygous wild-type), 8.0% had 1 mutation (heterozygotes), and 1.1% had 2 (homozygous null). Thirty-five individuals missing genotype data at 1 of the 2 loci were excluded from the primary combined analysis; inclusion of these subjects in the confirmatory analysis did not appreciably change our results (data not shown).

TABLE I. Phenotypic characteristics of subjects†

Variable	AD (N = 185)	No AD (N = 461)
Age (y)*	8.5 ± 2.2	9.0 ± 2.1
Male sex	115 (62%)	274 (59%)
IgE (IU/L)*	577 (202, 1487)	378 (136, 971)
Positive skin test*	168 (91%)	386 (84%)
Postalbuterol FEV ₁ (L)	1.8 ± (0.5)	1.8 ± (0.5)
FEV ₁ /FVC	0.81 ± (0.08)	0.79 ± (0.08)
Methacholine PC ₂₀ (mg/mL)	0.9 (0.4, 2.6)	1.1 (0.5, 2.8)

**P* < .05 for comparison between subjects with and without AD by using Fisher exact test (skin test) or Wilcoxon rank-sum (age and IgE).

†All values in mean ± SD, count (percentage), or median (interquartile range).

Association

Consistent with previous studies, *FLG* mutations in the CAMP cohort were associated with increased odds of AD (Table II). Also consistent with previous studies was the apparent semidominant genetic effect conferred by the mutations, with AD detected in 27% of wild-type homozygous subjects, 37% of heterozygotes, and 100% of mutant homozygotes. Case-control studies in genetics occasionally give false-positive results because of population stratification, a form of selection bias whereby unrecognized differences in genetic ancestry (rather than true biological differences) between cases and controls result in significant differences in genotype frequencies that are not related to disease. Family-based association testing is immune to population stratification, because it uses parental genotype data to control for this spurious admixture. A positive association in the context of family-based analysis is noted when there is a deviation from the random transmission expected under mendelian inheritance (ie, when the rate of transmitted to untransmitted alleles deviates from 1:1). In our study, family-based testing confirmed the association between atopic dermatitis and *FLG* mutations with significant overtransmission of the minor alleles in both the 2282del4 (transmitted:untransmitted ratio, 15:4; *P* = .01) and combination (transmitted:untransmitted ratio, 24:10; *P* = .02) mutations among subjects with AD (Table III).

In contrast with the strong associations observed with AD, *FLG* mutations were not associated with asthma. Because this was a case-only cohort, asthma affection status could be evaluated only in family-based analysis. Despite a suggestion of overtransmission of minor alleles of both the 2282del4 and combination mutations (with *P* values for asthma of .04 and .09, respectively), stratification of the sample by history of AD demonstrates that this apparent overtransmission is entirely driven by the subset of individuals with AD. As shown in Table III, among those subjects without concurrent AD, there is balanced allelic transmission, with transmission to nontransmission ratios for the minor alleles of 7:9, 11:9, and 17:17 for the R501X, 2282del4, and combination mutations, respectively.

Three measures of asthma severity were assessed in both family-based and population analysis. Subjects with and without *FLG* mutations did not differ in terms of reactivity to methacholine, postalbuterol FEV₁, or FEV₁/FVC ratio (Table IV). Even the subset with AD showed no trend toward a difference in lung function (Table V).

Although not associated with lung function variables, *FLG* mutations were associated with markers of atopy, particularly among individuals with AD. Family-based testing showed *FLG* mutations to be associated with a higher IgE level and an increased risk of skin test reactivity to ≥1 allergen among individuals with AD (Table V). These associations are attenuated when all subjects are considered, with only borderline IgE elevation by population testing (Table IV).

DISCUSSION

Two primary motivations for investigating the genetic basis of complex diseases are to identify novel biologic factors for pharmacologic targeting and to develop robust prognostic and diagnostic clinical applications. Given the extraordinary consistency in the association of *FLG* mutations with AD across diverse populations, it is clear that this locus substantially contributes to the population risk of AD and could serve both as a biologic target for future therapy and as a diagnostic and prognostic marker for AD specifically.

The question remains whether *FLG* mutations confer risk for the development of asthma or influence the natural history or severity of asthma beyond the indirect effects mediated by AD. Although previous studies have suggested such a relationship, they could not clearly address this question because the subjects with asthma studied were primarily ascertained on the basis of a diagnosis of AD. In contrast, the primary objective of our study was to examine the role of *FLG* mutations in asthma and asthma-related phenotypes where proband ascertainment was through a stringent diagnosis of asthma, without regard to AD affection status. This important difference in study design provided the opportunity to determine clearly whether *FLG* confers asthma risk independent of AD. As described, our analysis demonstrates that although *FLG* mutations confer strong risk for the development of AD in this cohort, they have no effect on the development of asthma or its severity if AD is not first established. *FLG* mutations demonstrated balanced transmission from parents to offspring with asthma without AD (50% transmission rate), and there was no evidence of association of *FLG* mutations with lung function or airway hyperresponsiveness, either in family-based or in population-based analysis. We also stress that even among those subjects with AD, we found no association of *FLG* mutations with lung function or airway responsiveness, providing further evidence that although these *FLG* mutations are important determinants of AD, they do not independently affect asthma risk.

TABLE II. Filaggrin mutations and AD

Genotype	2282del4		R501X		Combined genotype	
	No AD	AD	No AD	AD	No AD	AD
Wild-type homozygote	428	165	436	166	406	149
Heterozygote	21	12	11	12	31	18
Mutant homozygote	0	3	0	1	0	7
OR	2.0 (1.1-3.7)		3.1 (1.4-6.8)		2.4 (1.5-3.9)	
Fisher exact <i>P</i> value	.02		.01		7.6×10^{-5}	

Inadequate statistical power from small sample size or low disease allele frequency is a common cause of false-negative results (type II error) in studies of genetic association. Could our findings be a consequence of low statistical power? Despite studying a relatively large cohort of children with asthma ($n = 646$), the combined minor allele frequency of the *FLG* mutations was low (.05), resulting in only 60% power to demonstrate association with asthma affection status if an OR of 2.0 for asthma (similar to that for AD) is assumed. However, there are several reasons why we are confident that our observations are not a result of low power. First, power was reduced specifically because the allele frequency of *FLG* mutations in this asthma-ascertained cohort was only .05, similar to the population prevalence of controls in previous studies, but much lower than the reported frequencies among subjects with AD (.09-.34).^{1,5,7} This similarity between the carrier frequency of *FLG* mutations among non-AD subjects with asthma to the frequency among historical controls suggests that *FLG* mutations are not overrepresented (and thus not associated) with asthma. We further note that, had the carrier frequency in our cohort been similar to those in the AD cohorts, our power with this sample size would have been adequate (86% for an OR of 2.0). As a related point, because the minor allele frequency is low among subjects with asthma without AD, even if there is a modest genetic association, the resulting PAR of *FLG* mutations for asthma would be extremely low, in contrast with estimates of *FLG* mutation PAR for AD of 11%.⁵ Low statistical power affects only the ability to claim a statistically significant difference in disease risk between genotypes but does not in any way influence absolute risk. In our study, there was utterly no difference in transmission of *FLG* mutations from parent to offspring with asthma (ratio of transmission to nontransmission of 17:17; Table III). This suggests there was no independent effect of *FLG* mutations on asthma susceptibility, no matter how large a sample was studied or how high the minor allele frequency. Finally, although power was modest for detecting an association with asthma, we note that we had ample power for the quantitative analysis of asthma severity: we achieved 80% power to detect associations with lung function or airway responsiveness, even if the *FLG* mutations conferred only modest locus-specific heritability (as low as .04). Thus, our claims that *FLG* mutations do not contribute to asthma severity cannot be explained by reduced statistical power. We note that Sandilands et al¹⁶ recently reported 13 novel

TABLE III. Family-based association of filaggrin mutations with asthma affection, stratified by AD status*

	All subjects	Asthma + AD	Asthma, no AD
R501X	18:17 (.87)	11:8 (.49)	7:9 (.62)
2282del 4	26:13 (.04)	15:4 (.01)	11:9 (.65)
Combined genotype	41:27 (.09)	24:10 (.02)	17:17 (1.0)

*Represented as ratio of transmitted:untransmitted *FLG* minor alleles (*P* value).

missense and frameshift mutations in patients with severe ichthyosis vulgaris; several were shown to confer atopic dermatitis risk. Because most of these mutations are quite rare (several could be considered private mutations to 1 family), we feel that their inclusion in this study would not have meaningfully changed our power or results. Phenotypic misclassification is another common cause of false-negative genetic association. The current study is relatively immune to this risk because of the extensive phenotyping and stringent ascertainment criteria used for recruitment in the initial CAMP clinical trial. To be enrolled, subjects were required to have both physician-diagnosed asthma and documented airway hyperresponsiveness, in addition to evidence of persistent disease, as manifest by at minimum a 6-month history of either daily asthma medication use or at least twice weekly symptoms or medication use. Moreover, all lung function and airway responsiveness measurements used for the analysis presented here were obtained after a 28-day period when all subjects were off antiasthma controller therapy, thus excluding the possibility that our inability to demonstrate a genetic association with these measures was confounded by medication use. In contrast, we note strong associations with AD despite the fact that affection status of AD was established solely by parental report. That such compelling genetic association was noted by using such a weak phenotype definition suggests that the genetic effect of *FLG* mutations on the development of AD is indeed strong. We note that our conclusions about filaggrin and modulation of asthma and its severity differ markedly from those of Palmer et al,¹⁷ who examined 874 Scottish subjects with asthma age 3 to 22 years participating in the BREATHE study and concluded that *FLG* mutations were associated with greater asthma severity, as reflected by lower FEV₁/FVC ratios and greater asthma controller medication use. In comparing the 2 studies, we note

TABLE IV. Results of association analysis of filaggrin mutations with asthma-related intermediate phenotypes in CAMP probands of self-reported white ancestry*

	Homozygous wild-type (n = 555)	Wild-type/ mutant (n = 49)	Homozygous mutant (n = 7)	Test of association <i>P</i> value	
				Population-based analysis (n = 611)	Family-based analysis (n = 460 families)
AD, no. (%)	149 (27%)	18 (36%)	7 (100%)	7.6×10^{-5}	.03
IgE (IU/L), median (IQR)	396 (157, 1028)	712 (136, 1453)	779 (280, 4047)	.04	.26
Any positive skin test, no. (%)	484 (87%)	38 (78%)	7 (100%)	.14	.15
Postalbuterol FEV ₁ (L), mean $\pm$ SD	1.8 $\pm$ 0.5	1.8 $\pm$ 0.5	1.6 $\pm$ 0.5	.42	.47
Methacholine PC ₂₀ (mg/mL), median (IQR)	1.1 (0.5, 2.9)	1.2 (0.5, 2.1)	0.4 (0.3, 3.9)	.47	.98
FEV ₁ /FVC ratio, mean $\pm$ SD	.80 $\pm$.08	.80 $\pm$.08	.79 $\pm$.11	.56	.35

IQR, Interquartile range.

*For all analyses, IgE and PC₂₀ methacholine were log-transformed to achieve normality. Population-based analysis was performed by using linear models in SAS v.9.1. Family-based analysis was performed by using PBAT.^{13,14} Quantitative trait analysis was adjusted for age and sex (IgE and FEV₁/FVC) and for height (FEV₁ and PC₂₀). Associations with combined loss-of-function genotype presented. Analysis of individual mutations produced similar results (data not shown).

TABLE V. Results of association analysis of filaggrin mutations with asthma-related intermediate phenotypes in white CAMP probands, restricted to subjects with AD*

	Homozygous wild-type (n = 149)	Wild-type/ mutant (n = 18)	Homozygous mutant (n = 7)	Test of association <i>P</i> value	
				Population-based analysis (n = 174)	Family-based analysis (n = 143 families)
IgE (IU/L), median (IQR)	456 (193, 1400)	1025 (523, 1453)	779 (280, 4047)	.06	.009
Any positive skin test, no. (%)	138 (93%)	16 (89%)	7 (100%)	.79	.02
Postalbuterol FEV ₁ (L), mean $\pm$ SD	1.8 $\pm$ 0.6	1.6 $\pm$ 0.4	1.6 $\pm$ 0.5	.12	.15
Methacholine PC ₂₀ (mg/mL), median (IQR)	0.8 (0.4, 2.6)	0.9 (0.7, 1.5)	0.4 (0.3, 3.9)	.72	.60
FEV ₁ /FVC ratio, mean $\pm$ SD	.80 $\pm$.08	.82 $\pm$.06	.79 $\pm$.11	.69	.25

IQR, Interquartile range.

*For all analyses, IgE and PC₂₀ methacholine were log-transformed to achieve normality. Population-based analysis was performed by using linear models in SAS v.9.1. Family-based analysis was performed by using PBAT.^{13,14} Quantitative trait analysis was adjusted for age and sex (IgE and FEV₁/FVC) and for height (FEV₁ and PC₂₀). Associations with combined loss-of-function genotype presented. Analysis of individual mutations produced similar results (data not shown).

several important methodological differences relating to sample ascertainment, phenotype measurement, and data interpretation that may account for these diverging conclusions. First, with regard to ascertainment, we note that the sample used in this recent BREATHE analysis represents an extension of that included in the initial report describing association of *FLG* with AD, with nearly 2/3 of the sample used in the current analysis having contributed to the initial analysis. Thus, in the context of a *post hoc* analysis of *FLG* mutations as they relate to asthma severity, the BREATHE sample cannot be considered an independently ascertained sample, given that populations contributing to initial reports of association frequently overestimate the magnitude of genetic effects (the so-called “winners-curse”).^{18,19} Supporting this is the greater representation in BREATHE (as compared with CAMP) of *FLG* mutations (carrier frequencies of 13% vs 8.6%, respectively) and of AD (55% vs 29%). Second, with regard to phenotype measurement, we note that, unlike in CAMP, lung function measurements in BREATHE were obtained while on antiasthma therapy. Third, with regard to data interpretation, the most

significant relationships of *FLG* mutations with asthma severity in the BREATHE cohort were obtained in an analysis of all subjects, regardless of AD status, whereas analyses restricted to subjects with asthma without AD produced much less significant results ($P = .074$). Although this suggests a trend of association, we find it difficult to conclude from these data that *FLG* mutations affect asthma severity independent of AD status. Finally, we note that the prevalence of *FLG* mutations among the subjects with asthma without AD was similar to that of the general population in Tayside, Scotland, with an excess of *FLG* mutations observed only among subjects with AD, suggesting no genetic association of *FLG* mutations with asthma in the absence of AD—findings in keeping with those of our study.

In summary, our findings again confirm that *FLG* mutations are an important, common prognostic factor for AD. In contrast, although it is well established that AD is associated with increased asthma severity and greater asthma persistence into adulthood,^{20,21} and it is certainly possible that filaggrin mutations may modulate asthma severity indirectly in patients with AD (by impairing the

normal skin barrier function and resulting in increased allergen presentation), our data demonstrate that filaggrin does not influence the development of asthma or affect its severity in the absence of concomitant AD. Future work on filaggrin as a potential therapeutic target should focus on atopic dermatitis, not asthma *per se*.

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REFERENCES

- Palmer CN, Irvine AD, Terron-Kwiatkowski A, Zhao Y, Liao H, Lee SP, et al. Common loss-of-function variants of the epidermal barrier protein filaggrin are a major predisposing factor for atopic dermatitis. *Nat Genet* 2006;38:441-6.
- Smith FJ, Irvine AD, Terron-Kwiatkowski A, Sandilands A, Campbell LE, Zhao Y, et al. Loss-of-function mutations in the gene encoding filaggrin cause ichthyosis vulgaris. *Nat Genet* 2006;38:337-42.
- Weidinger S, Illig T, Baurecht H, Irvine AD, Rodriguez E, Diaz-Lacava A, et al. Loss-of-function variations within the filaggrin gene predispose for atopic dermatitis with allergic sensitizations. *J Allergy Clin Immunol* 2006;118:214-9.
- Barker JN, Palmer CN, Zhao Y, Liao H, Hull PR, Lee SP, et al. Null mutations in the filaggrin gene (FLG) determine major susceptibility to early-onset atopic dermatitis that persists into adulthood. *J Invest Dermatol* 2007;127:564-7.
- Marenholz I, Nickel R, Ruschendorf F, Schulz F, Esparza-Gordillo J, Kerscher T, et al. Filaggrin loss-of-function mutations predispose to phenotypes involved in the atopic march. *J Allergy Clin Immunol* 2006;118:866-71.
- Ruether A, Stoll M, Schwarz T, Schreiber S, Folster-Holst R. Filaggrin loss-of-function variant contributes to atopic dermatitis risk in the population of Northern Germany. *Br J Dermatol* 2006;155:1093-4.
- Stemmler S, Parwez Q, Petrasch-Parwez E, Epplen JT, Hoffjan S. Two common loss-of-function mutations within the filaggrin gene predispose for early onset of atopic dermatitis. *J Invest Dermatol* 2007;127:722-4.
- Weidinger S, Rodriguez E, Stahl C, Wagenpfeil S, Klopp N, Illig T, et al. Filaggrin mutations strongly predispose to early-onset and extrinsic atopic dermatitis. *J Invest Dermatol* 2007;127:724-6.
- Irvine AD. Fleshing out filaggrin phenotypes. *J Invest Dermatol* 2007;127:504-7.
- Ying S, Meng Q, Corrigan CJ, Lee TH. Lack of filaggrin expression in the human bronchial mucosa. *J Allergy Clin Immunol* 2006;118:1386-8.
- The Childhood Asthma Management Program (CAMP): design, rationale, and methods. Childhood Asthma Management Program Research Group. *Control Clin Trials* 1999;20:91-120.
- Long-term effects of budesonide or nedocromil in children with asthma. The Childhood Asthma Management Program Research Group. *N Engl J Med* 2000;343:1054-63.
- Lange C, DeMeo D, Silverman EK, Weiss ST, Laird NM. Using the non-informative families in family-based association tests: a powerful new testing strategy. *Am J Hum Genet* 2003;73:801-11.
- Lange C, Laird NM. On a general class of conditional tests for family-based association studies in genetics: the asymptotic distribution, the conditional power, and optimality considerations. *Genet Epidemiol* 2002;23:165-80.
- Barrett JC, Fry B, Maller J, Daly MJ. Haploview: analysis and visualization of LD and haplotype maps. *Bioinformatics* 2005;21:263-5.
- Sandilands A, Terron-Kwiatkowski A, Hull PR, O'Regan GM, Clayton TH, Watson RM, et al. Comprehensive analysis of the gene encoding filaggrin uncovers prevalent and rare mutations in ichthyosis vulgaris and atopic eczema. *Nat Genet* 2007;39:650-4.
- Palmer CN, Ismail T, Lee SP, Terron-Kwiatkowski A, Zhao Y, Liao H, et al. Filaggrin null mutations are associated with increased asthma severity in children and young adults. *J Allergy Clin Immunol* 2007;120:64-8.
- Ioannidis JP, Ntzani EE, Trikalinos TA, Contopoulos-Ioannidis DG. Replication validity of genetic association studies. *Nat Genet* 2001;29:306-9.
- Lohmueller KE, Pearce CL, Pike M, Lander ES, Hirschhorn JN. Meta-analysis of genetic association studies supports a contribution of common variants to susceptibility to common disease. *Nat Genet* 2003;33:177-82.
- Martinez FD, Wright AL, Taussig LM, Holberg CJ, Halonen M, Morgan WJ. Asthma and wheezing in the first six years of life. The Group Health Medical Associates. *N Engl J Med* 1995;332:133-8.
- Strachan DP, Butland BK, Anderson HR. Incidence and prognosis of asthma and wheezing illness from early childhood to age 33 in a national British cohort. *BMJ* 1996;312:1195-9.