

Filaggrin polymorphism P478S, IgE level, and atopic phenotypes

I.J. Wang,*†‡ T.J. Lin,§ C.F. Kuo,¶ S.L. Lin,¶ Y.L. Lee** and P.C. Chen**

*Department of Pediatrics, Taipei Hospital, Department of Health, Taipei, Taiwan

†College of Public Health, China Medical University, Taichung, Taiwan

‡College of Medicine, Fu Jen Catholic University, Taipei, Taiwan

§Wan Fang Hospital, Taipei Medical University, Taipei, Taiwan

¶Taipei Hospital, Department of Health, Taipei, Taiwan

**Institute of Occupational Medicine and Industrial Hygiene, National Taiwan University College of Public Health, 17 Syujhou Road, Taipei 10055, Taiwan

Summary

Correspondence

Pau-Chung Chen.

E-mail: pchen@ntu.edu.tw; r92846001@ntu.edu.tw

Accepted for publication

28 December 2010

Funding sources

This study was supported by grants from the National Science Council (NSC 97-2314-B-192-001-MY2) and Taipei Hospital, Department of Health in Taiwan.

Conflicts of interest

None declared.

DOI 10.1111/j.1365-2133.2011.10212.x

Background Whether environmental exposures may modulate the effect of the skin barrier gene on atopic dermatitis (AD) remains to be elucidated.

Objectives To determine whether filaggrin (FLG) variants can serve as a predictor for atopic disorders in Chinese individuals and if allergen exposures may modify the effect of FLG variants on AD by total IgE levels.

Methods In total, 116 children aged 2–5 years with AD and 212 control subjects were analysed for the FLG variants using DNA sequencing. Multiple logistic regression models were performed to estimate the association among FLG polymorphisms and atopic phenotypes. Serum total IgE level, standing for the degree of allergen exposures, was later stratified to determine the effects of FLG polymorphisms on AD.

Results A significant difference in genotype frequency was found among AD cases and controls in FLG P478S polymorphism. FLG P478S GG genotype significantly increased the risk of AD [odds ratio (OR) 4.60, 95% confidence interval (CI) 1.88–11.24]. In addition, among subjects with AD, GG genotypes also significantly increased the risk of developing asthma (OR 4.68, 95% CI 1.37–16.03). Further, a similar result was obtained for allergic rhinitis (OR 3.23, 95% CI 1.01–10.30). Interestingly, the P478S GG genotype was significantly related to AD (OR 5.67, 95% CI 1.93–16.60) in children with IgE level ≥ 100 kU L⁻¹. However, the association was not evident when IgE level was < 100 kU L⁻¹.

Conclusions Our results suggest that the FLG P478S polymorphism may confer susceptibility to the development of AD among Chinese individuals and may be modified by IgE levels.

Atopic dermatitis (AD) is a debilitating chronic relapsing inflammatory skin disorder that is highly prevalent in the developed world, where it affects 15–20% of children.¹ Because most infants with AD are most likely to develop asthma later in life, many researchers have previously focused their genetic analysis on asthma susceptibility regions. However, genome-wide studies have shown merely partial overlap of AD with asthma, but a closer relationship with other epithelial diseases.² Therefore, awareness has been drawn to the impaired skin barrier function which is a hallmark of AD. Although changes in three groups of genes encoding structural proteins, epidermal proteases and protease inhibitors predispose to a defective epidermal barrier, the powerful effect of filaggrin

(FLG) gene variation on AD risk exceeds that of any other investigated candidate gene thus far.³

FLG is located within the epidermal differentiation complex (EDC) 1q21. The EDC is a dense cluster of genes involved in the terminal differentiation of the epidermis and formation of the stratum corneum. FLG is a key epidermal protein in facilitating the terminal differentiation of keratinocytes and thereby in maintaining normal skin barrier function and hydration.⁴ To date, approximately 40 loss of-function FLG mutations have been identified in AD.^{5,6} In the European population, up to six recurrent FLG mutations and several other family-specific mutations have been discovered. Among them, the two mutations R501X and 2282del4 are the most prevalent.^{7,8}

However, Nomura *et al.*⁹ found that none of the investigated Japanese ichthyosis vulgaris families carried the European-specific mutations and identified four novel mutations, S2554X, 3321delA, S2889X and S3296X, to be strongly associated with AD in the Japanese cohort. Subsequently, two additional mutations, Ser1695X and Gln1701X, were detected.¹⁰ A novel FLG nonsense mutation, Lys4021X, was also identified in one patient with AD without any other known Japanese FLG mutation.⁶ Furthermore, one of the common European mutations, Arg501X, was reported in a Japanese family.¹¹ Overall, there are at least eight FLG variants in the Japanese population. In the Chinese population, Ma *et al.*¹² reported that FLG mutation 3321delA is a risk factor for a subset of patients with AD, particularly in males with concomitant respiratory allergic diseases and early age at onset. Moreover, Ching *et al.*¹³ detected FLG mutation R501X in Chinese children with AD. Most FLG variants are specific to different populations; nevertheless, FLG variants in the Taiwanese population have not been fully analysed.

As the skin barrier plays a crucial role in preventing allergic sensitization,¹⁴ understanding of gene–environment interactions in AD will provide a rationale for targeted therapeutics capable of preventing disruption of barrier function. However, whether skin barrier function in individuals with FLG variants may modulate the effect of environmental exposures, thus modifying the expression of AD, remains to be elucidated. In this study, we wished to evaluate if FLG variants can serve as a predictor for atopic disorders in Chinese individuals. Moreover, we also investigated if allergen exposures may modify the effect of FLG variants on AD by total IgE levels.

Materials and methods

Study population

In total, 116 children aged 2–5 years with AD were recruited from hospital-based clinics. Two control subjects selected from well baby clinics and the Taiwan birth panel study as described elsewhere¹⁵ were matched to each case subject by gender and age (within 5 months). Finally, 212 control specimens were available. DNA samples were collected with institutional research ethics committee approval at our hospital and informed consent that complies with all the Declaration of Helsinki Principles. All subjects were of the same ethnic origin.

Case definition

AD cases were defined according to the diagnostic criteria of Hanifin and Rajka.¹⁶ Children not included in these categories were classified as control subjects. Detailed phenotypic data were gathered and examined by experienced physicians. Early-onset AD was defined as age at onset of AD < 2 years. The association between FLG mutations and asthma and allergic rhinitis was investigated as a secondary analysis. Children were considered as having asthma if the answer to the question ‘Has a doctor ever diagnosed this child as having asthma?’ was ‘yes’

in a validated International Study of Asthma and Allergies in Childhood (ISAAC) questionnaire. The definition of allergic rhinitis was also based on the parent’s information of a doctor’s diagnosis of allergic rhinitis in the ISAAC questionnaire.

Laboratory methods

IgE antibody analysis

Total serum IgE concentrations were determined with Pharmacia UniCap IgE assay test system (Pharmacia Diagnostics, Uppsala, Sweden). IgE levels were considered increased at values of ≥ 100 kU L⁻¹. Concentrations < 0.35 kU L⁻¹ were defined as absent or undetectable IgE.

Genotyping

Genomic DNA was extracted from oral epithelial cells and blood leucocytes by a commercially available DNA isolation kit (Puregene; Gentra Systems, Minneapolis, MN, U.S.A.). The variants of genetic candidates, including common polymorphisms (T454A, P478S, E498D, H519N, R3270C, Q3322Q) and rare mutations (R501X, S3247X, S3296X, S2554X, S2889X, 2282del4, 3321delA, E1795X, E2422X and Q2417X) (Table S1, see Supporting Information) selected from hotspots of Asian populations from the NCBI website and literature reviews, were performed by DNA sequencing as described elsewhere.^{7–9,17} The primer sequences (Table S2; see Supporting Information) were designed by Primer3 v0.4.0 (<http://frodo.wi.mit.edu/primer3/>) and synthesized from Integrated DNA Technologies (Coralville, IA, U.S.A.). Polymerase chain reaction (PCR) was performed in a total volume of 20 μ L containing 50 mmol L⁻¹ KCl, 10 mmol L⁻¹ Tris–HCl (pH 8.3), 5–10% dimethylsulphoxide, 1.5 mmol L⁻¹ MgCl₂, 200 mmol L⁻¹ each of dATP, dCTP, dGTP and dTTP, 0.5–1.0 U of Super-Therm polymerase, 20 pmol of each primer, and 50 ng of gDNA. The PCR amplification conditions were 95 °C for 30 s, 60–65 °C for 30 s, and 72 °C for 30 s for 40 cycles followed by 10 min at 72 °C in an Eppendorf 5331 thermal cycler (Eppendorf, Hamburg, Germany). The PCR products were analysed with ethidium bromide–agarose electrophoresis in Tris–acetate–EDTA buffer. Then they were cleaned up with exonuclease I (New England Biolabs, Ipswich, MA, U.S.A.) and shrimp alkaline phosphatase (Promega, Madison, WI, U.S.A.). The ExoSAP PCR products were used as templates to perform DNA sequencing reactions. The reactions were carried out by Big Dye Terminator v3.1 Cycle Sequencing kit (Applied Biosystems, Foster City, CA, U.S.A.) and capillary electrophoresis on an ABI 3730 DNA Sequencer (Applied Biosystems). Sequencing data were analysed with DNA Sequencing Analysis Software v5.2 (Applied Biosystems) and Codoncode Aligner Software v2.0.6 (Codoncode, Dedham, MA, U.S.A.). The quality of DNA sequencing was monitored with three quality assurance guarantees including average signal intensity > 300, the ratio of signal/noise > 50, and the QV value (the established metric for determining the

quality of sequencing data) > 20 (meaning an error rate of $\leq 1\%$). All groups compared were measured in the same assay in duplicate for each subject to reduce the influence of unavoidable interassay variances. To control for errors and technical problems in genotyping, derived genotype frequencies were compared with the expected allelic population equilibrium based on the Hardy–Weinberg equilibrium test.

Statistical analysis

Baseline characteristics were compared between case and control subjects with two-sample t-test for continuous variables or χ^2 tests for categorical variables. Allele and genotype frequencies were compared using χ^2 tests. We further investigated the subset of children with AD and evaluated the FLG variants as a risk factor for asthma and allergic rhinitis by Fisher exact tests. To study the modification effect of IgE, we divided IgE levels into two categories with $< 100 \text{ kU L}^{-1}$ standing for low allergen exposures and $\geq 100 \text{ kU L}^{-1}$ standing for high allergen exposures, with the lower level used as the reference category.¹⁴ For the association between IgE levels and FLG polymorphisms with AD, odds ratios (ORs) with 95% confidence intervals (CIs) were calculated by conditional logistic regression models. Analyses were performed in both univariate and multivariate models. Next, we tested for gene–environment interaction by adding a product term in the regression model. Family income, maternal age, maternal education, maternal history of atopy, duration of breast feeding, birth weight, number of older siblings, usage of carpets or incense at home, environmental tobacco exposure, and fungi on house walls were all taken into consideration as potential confounders for analysis. Those that made 10% change in point estimates in the statistical procedures were then adjusted for. All hypothesis testing was two-sided at the significance level of 0.05 and was performed with SAS software v9.1 (SAS Institute, Cary, NC, U.S.A.).

Results

Of the 116 AD cases, 52.6% had asthma, 47.4% had allergic rhinitis, and 73.3% showed an early onset of the disease. The total IgE of AD cases was higher than that of controls (mean $\pm$ SD 210.08 ± 372.76 vs. $124.12 \pm 215.58 \text{ kU L}^{-1}$, $P = 0.009$). The demographic characteristics are outlined in Table 1. There was no significant difference between cases and controls with the exception of maternal history of atopy.

The European-specific mutations R501X and 2282del4 and Japanese-specific mutations S2554X, S2889X and S3296X were absent in Chinese individuals. However, a significant difference in genotype frequency was found among AD cases and controls in FLG P478S (rs11584340) polymorphism. Observed genotype frequencies did not deviate significantly from expected frequencies under the Hardy–Weinberg equilibrium test assumption. The frequency of the GG genotype in patients with AD was significantly higher than in controls (14.7% vs. 3.8%, $P = 0.002$) (Table 2). The G allele frequency of patients (31.0%) was also significantly higher than that of controls

Table 1 Characteristics of the study population

Characteristics	Cases (n = 116)	Controls (n = 212)
Mother		
Maternal age at delivery (years), median (range)	30 (20–40)	28 (22–37)
Maternal education, n (%)		
High school and below	47 (40.5)	106 (50.0)
College and above	69 (59.5)	106 (50.0)
Maternal history of atopy, n (%)	24 (20.7)*	17 (8.0)*
Children		
Gestational age < 37 weeks, n (%)	3 (7.0)	16 (8.2)
Birth weight (g), mean $\pm$ SD	3196.0 $\pm$ 395.2	3180.6 $\pm$ 467.1
Environmental factors		
Duration of breast feeding < 6 months, n (%)	48 (41.4)	100 (47.2)
Two or more older siblings, n (%)	54 (46.6)	114 (53.8)
Fungi on house wall, n (%)	47 (40.5)	100 (47.2)
Carpets at home, n (%)	4 (3.5)	15 (7.1)
Incense used at home, n (%)	62 (53.5)	100 (47.2)
ETS exposure, n (%)	50 (43.1)	78 (36.8)
Family income per year (NT dollars), n (%)		
< 600 000	27 (23.3)	37 (17.5)
600 000–1 500 000	68 (58.6)	129 (60.8)
>1 500 000	21 (18.1)	46 (21.7)

ETS, environmental tobacco smoke; NT dollars, New Taiwan dollars. * $P < 0.05$.

(20.8%) ($P = 0.0034$). Carriers of the GG genotype were more likely than noncarriers to have AD (adjusted OR 4.50, 95% CI 1.84–11.03) (Table 3).

We further investigated the subset of children with AD. AD subjects with P478S GG genotype had a significantly increased risk (OR 4.68, 95% CI 1.37–16.03) of developing asthma compared with those carrying AA or AG genotypes (Table 4). Similar results were obtained for allergic rhinitis (OR 3.23, 95% CI 1.01–10.30) and the early-onset phenotype (OR 4.26, 95% CI 0.49–37.26).

We also investigated the effect of FLG polymorphisms and allergen exposures on AD by total IgE levels (Table 3). In children with IgE level $\geq 100 \text{ kU L}^{-1}$, FLG P478S GG genotype was significantly related to AD (OR 5.67, 95% CI 1.93–16.60). However, the association was not evident when IgE level was $< 100 \text{ kU L}^{-1}$. Subsequently, we tried to assess the interaction effects between FLG P478S GG genotype and total IgE levels in AD subjects. However, the analyses of FLG P478S GG genotype and total IgE levels did not show significant interactive effects.

Discussion

This study is a contribution to the literature on the potential association among FLG genetic polymorphisms, IgE and AD.

P478S	AA, n (%)	AG, n (%)	GG, n (%)	P-value	Allele frequency		
					A, n (%)	G, n (%)	P-value
Cases	61 (52.6)	38 (32.8)	17 (14.7)	0.002	160 (69.0)	72 (31.0)	0.0034
Controls	132 (62.3)	72 (34.0)	8 (3.8)		336 (79.3)	88 (20.8)	

Table 2 Allele and genotype frequencies of the FLG 478S polymorphism in the study population

FLG P478S, n (%)	Cases	Controls	Crude OR (95% CI)	Adjusted OR (95% CI) ^a
Total population				
AA	61 (52.6)	132 (62.3)	—	—
AG	38 (32.8)	72 (34.0)	1.14 (0.70–1.88)	1.08 (0.65–1.79)
GG	17 (14.7)	8 (3.8)	4.60 (1.88–11.24)*	4.50 (1.84–11.03)*
IgE level < 100 kU L ⁻¹				
AA	16 (61.5)	47 (60.3)	—	—
AG	8 (30.8)	28 (35.9)	0.84 (0.32–2.21)	0.73 (0.27–2.00)
GG	2 (7.7)	3 (3.8)	1.96 (0.30–12.80)	1.96 (0.30–12.80)
IgE level ≥ 100 kU L ⁻¹				
AA	45 (50.0)	85 (63.4)	—	—
AG	30 (33.3)	44 (32.8)	1.29 (0.72–2.32)	1.24 (0.68–2.52)
GG	15 (16.7)	5 (3.7)	5.67 (1.93–16.60)*	5.58 (1.90–16.37)*

^aModel adjusted for maternal history of atopy. OR, odds ratio; CI, confidence interval.

*P < 0.05.

Table 3 The association between FLG P478S polymorphism and atopic dermatitis stratified by IgE levels

Our results further reinforce the role of the FLG P478S polymorphism on AD, which is the strong genetic factor identified in this common complex disease. To the best of our knowledge, the interaction between FLG genotypes and IgE levels in subjects with AD has not been studied before. We found that the adverse effects of allergen exposures were largely restricted to children carrying the FLG P478S GG genotype.

Combining our results with the published data, the European mutations R501X and 2282del4 and Japanese-specific mutations S2554X, S2889X and S3296X are either extremely rare or, more likely, completely absent within the Chinese populations.^{7,9,13} We identify, however, one specific FLG P478S polymorphism in the Taiwanese Chinese children with AD. Although differences exist in the spectra of FLG mutations

observed among different ethnic groups, FLG mutations appear to be significant predisposing factors for AD universally. Therefore, it is important to establish global population genetic maps of FLG variants.

The polymorphism of FLG P478S played a role in the development of AD in our study. One of the explanations is that P478S is located in exon 3 of the FLG gene. It is at the initiating part of the first FLG repeat and near the first linker segment, codon 468–474, which connects the 5'-end with the truncated FLG repeat.¹⁸ The 478 serine residue may be phosphorylated and hampers the protease cleavage, thus affecting the aggregation rate between FLG and keratin filaments. Another possible explanation is that the FLG variant is associated with AD through linkage disequilibrium with another unrecognized gene which is the real susceptibility locus. However, this seems implausible because of the independent relationship of five different FLG mutations with AD in the European population and the reduction in FLG expression in atopic skin as a result of FLG mutations in some biochemical studies.^{18,19} It is possible that other genetic variants – serine protease inhibitor SPINK5 and kallikrein 7 gene KLK7 – involved in skin barrier function also modify the risk of developing AD. However, it was recently reported that neither KLK7 nor SPINK5 had any epistatic effects with FLG alleles.²⁰ Furthermore, there might be confounding effects due to population heterogeneity. Nevertheless, this effect was weak as our study subjects were all Taiwanese Chinese, a rather homogeneous population. Taken together, our study does support a significant role for FLG variants in the pathogenesis of this common complex disease.

Table 4 The association between FLG P478S polymorphism with atopic phenotypes restricted to subjects with atopic dermatitis (AD)

Genotype	Asthma (–)	Asthma (+)	AD			
			AR (–)	AR (+)	Early onset	Late onset
AA	36	25	35	26	39	22
AG	21	17	15	23	30	8
GG	4	13	5	12	16	1
Total	61	55	55	61	85	31
P-value	0.033		0.063		0.026	
OR (recessive)	4.68		3.23		4.26	
95% CI	1.37–16.03		1.01–10.30		0.49–37.26	

AR, allergic rhinitis; OR, odds ratio; CI, confidence interval.

As some of the children with FLG variants did not have any signs of AD, we hypothesize that other environmental factors may also play a role in allowing abnormally enhanced cutaneous presentation of antigens, allergens and chemicals to the immune system. We also demonstrated that allergen exposures could modify the risk of FLG variants on AD by IgE levels in our study. Although the reduced sample size might lower the statistical power to detect any interaction effect in our study, we did find various associations of FLG P478S GG genotype with AD stratified by IgE levels. This further supports the importance of environmental exposures to an impaired skin barrier. It was previously reported that topical application of allergen to mice homozygous for *Flg* mutation results in cutaneous inflammatory infiltrates and enhanced cutaneous allergen priming with development of allergen-specific antibody responses.²¹ Observations of children with AD and the FLG variants who were exposed to topical emollients containing peanut protein were in line with these findings. These children showed a significantly increased risk of peanut sensitization.²² These data all provided evidence that antigen transfer through a defective epidermal barrier interacting with antigen-presenting cells is a key mechanism underlying elevated IgE sensitization with FLG-related atopic disease.

Previous studies speculated that the disturbance of epidermal barrier in subjects with AD would subsequently lead to the development of asthma (so-called 'atopic march').²³ We also found associations of FLG variants with asthma and allergic rhinitis in subjects with AD. Therefore, our results further reinforce the relationship between FLG variants and respiratory atopic diseases. Even though the results in the ALSPAC (Avon Longitudinal Study of Parents and Children) and ISAAC II studies were limited to asthma in the context of AD, they further confirm the associations.^{24,25} Our data raise important questions in regard to mechanisms behind the association of a skin barrier gene with asthma. As FLG is not expressed in the bronchial mucosa, it does not appear to affect asthma pathogenesis directly by regulating allergen permeability in the bronchial mucosa.²⁶ Percutaneous priming and secondary immunological effects from the induction of Th2 cytokines in epithelia are interesting hypotheses that have been put forward.²⁷ Additional longitudinal population-based studies are needed to evaluate whether the association of FLG P478S polymorphism with asthma is independent of the association with AD. Our results also support a recent finding of the association between FLG variants and early-onset AD.²⁸ Percutaneous absorption of topically applied substances is higher in early infancy.²⁹ Combined with a higher surface area/weight ratio in infants, a genetically dysfunctional skin barrier is expected to be of greater significance in infants with AD. Genetic screening for FLG variants through the analysis of cord blood specimens could help identify individuals with early-onset atopic phenotypes for early prevention.

One of the strengths of our study was that single-nucleotide polymorphism (SNP) genotyping was performed using

DNA sequencing. The validity of DNA sequencing is higher than methods using restriction fragment length polymorphisms or quantitative PCR (TaqMan) assay. Furthermore, assessing allergen sensitization by serum IgE, a sensitive quantitative biomarker of allergen sensitization, can reduce the recall bias of allergen exposure assessment from questionnaires. We were also careful to define AD using a well-validated method (Hanifin and Rajka's diagnostic criteria) in addition to skin examination by an experienced physician. This may decrease the diagnosis bias. Our study has some potential limitations. As it was difficult to perform lung function tests and rhinoscopy examinations for young children, we used the ISAAC questionnaire for diagnosing asthma or allergic rhinitis. This could be a potential bias. Nevertheless, the ISAAC questionnaire has been previously validated in children against clinical investigation in studies performed in several European countries.^{30–32} Because the genetic architecture of FLG-related AD seems to consist of a mixture of prevalent FLG polymorphisms common within different races/ethnicities in addition to several infrequent, family-specific, and individualized mutations,³³ we cannot exclude the possibility that there are more FLG variants in the Chinese population yet to be discovered. However, some FLG variants reported may be rare mutations with low allele frequency in the populations, which might not be a sensitive screening tool.^{7,8} Based on the common disease–common variant hypothesis,³⁴ P478S was the most common variant of the FLG coding region in the SNP database of the NCBI and may serve as a good screening tool.¹² Further comprehensive analysis of the FLG gene in the Chinese population is ongoing in our laboratories to search for other candidate SNPs.

In conclusion, we have uncovered that FLG P478S polymorphism may confer susceptibility to the development of AD among Chinese individuals and predispose subjects with AD later to develop asthma and allergic rhinitis. As the adverse effects of allergen exposures were largely restricted to children carrying FLG P478S GG genotype, preventive measures to avoid allergens or early interventions using topical emollients to reconstitute the skin barrier function should be introduced in these susceptible children to avoid subsequent sensitization.

What's already known about this topic?

- Whether environmental exposures may modulate the effect of the skin barrier gene on atopic dermatitis (AD) remains to be elucidated.

What does this study add?

- FLG variants can serve as a predictor for AD in Chinese individuals.
- Allergen exposures may modify the effect of FLG variants on AD by total IgE levels.

Acknowledgments

We appreciate the assistance from Ms Su-lien Chen, Genomics BioSci & Tech, for laboratory analysis.

References

- Williams H, Flohr C. How epidemiology has challenged 3 prevailing concepts about atopic dermatitis. *J Allergy Clin Immunol* 2006; **118**:209–13.
- Cookson W. The immunogenetics of asthma and eczema: a new focus on the epithelium. *Nat Rev Immunol* 2004; **4**:978–88.
- Cork MJ, Danby SG, Vasilopoulos Y *et al.* Epidermal barrier dysfunction in atopic dermatitis. *J Invest Dermatol* 2009; **129**:1892–908.
- Mischke D, Korge BP, Marenholz I *et al.* Genes encoding structural proteins of epidermal cornification and S100 calcium-binding proteins form a gene complex ('epidermal differentiation complex') on human chromosome 1q21. *J Invest Dermatol* 1996; **106**:989–92.
- O'Regan GM, Sandilands A, McLean WH, Irvine AD. Filaggrin in atopic dermatitis. *J Allergy Clin Immunol* 2008; **122**:689–93.
- Nemoto-Hasebe I, Akiyama M, Nomura T *et al.* FLG mutation p.Lys4021X in the C-terminal imperfect filaggrin repeat in Japanese patients with atopic eczema. *Br J Dermatol* 2009; **161**:1387–90.
- Sandilands A, Terron-Kwiatkowski A, Hull PR *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.
- Akiyama M. FLG mutations in ichthyosis vulgaris and atopic eczema: spectrum of mutations and population genetics. *Br J Dermatol* 2010; **162**:472–7.
- Nomura T, Akiyama M, Sandilands A *et al.* Specific filaggrin mutations cause ichthyosis vulgaris and are significantly associated with atopic dermatitis in Japan. *J Invest Dermatol* 2008; **128**:1436–41.
- Nomura T, Akiyama M, Sandilands A *et al.* Prevalent and rare mutations in the gene encoding filaggrin in Japanese patients with ichthyosis vulgaris and atopic dermatitis. *J Invest Dermatol* 2009; **129**:1302–5.
- Hamada T, Sandilands A, Fukuda S *et al.* De novo occurrence of the filaggrin mutation p.R501X with prevalent mutation c.3321delA in a Japanese family with ichthyosis vulgaris complicated by atopic dermatitis. *J Invest Dermatol* 2008; **128**:1323–5.
- Ma L, Zhang L, Di ZH *et al.* Association analysis of filaggrin gene mutations and atopic dermatitis in Northern China. *Br J Dermatol* 2010; **162**:225–7.
- Ching GK, Hon KL, Ng PC, Leung TF. Filaggrin null mutations in childhood atopic dermatitis among the Chinese. *Int J Immunogenet* 2009; **36**:251–4.
- Weidinger S, Illig T, Baurecht H *et al.* Loss-of-function variations within the filaggrin gene predispose for atopic dermatitis with allergic sensitizations. *J Allergy Clin Immunol* 2006; **118**:214–19.
- Wang IJ, Hsieh WS, Guo YL *et al.* Neuro-mediators as predictors of paediatric atopic dermatitis. *Clin Exp Allergy* 2008; **38**:1302–8.
- Hanifin JM, Rajka G. Diagnostic features of atopic dermatitis. *Acta Derm Venereol (Stockh)* 1980; **92** (Suppl.):44–7.
- NCBI. Reference SNP Cluster Report. Available at: http://www.ncbi.nlm.nih.gov/projects/SNP/snp_ref.cgi?rs=11584340 (last accessed 30 January 2011).
- Presland RB, Haydock PV, Fleckman P *et al.* Characterization of the human epidermal profilaggrin gene. Genomic organization and identification of an S-100-like calcium binding domain at the amino terminus. *J Biol Chem* 1992; **267**:23772–81.
- Seguchi T, Cui CY, Kusuda S *et al.* Decreased expression of filaggrin in atopic skin. *Arch Dermatol Res* 1996; **288**:442–6.
- Weidinger S, Baurecht H, Wagenpfeil S *et al.* Analysis of the individual and aggregate genetic contributions of previously identified serine peptidase inhibitor Kazal type 5 (SPINK5), kallikrein-related peptidase 7 (KLK7), and filaggrin (FLG) polymorphisms to eczema risk. *J Allergy Clin Immunol* 2008; **122**:560–8.
- Fallon PG, Sasaki T, Sandilands A *et al.* A homozygous frameshift mutation in the mouse Flg gene facilitates enhanced percutaneous allergen priming. *Nat Genet* 2009; **41**:602–8.
- Lack G, Fox D, Northstone K, Golding J. Factors associated with the development of peanut allergy in childhood. *N Engl J Med* 2003; **348**:977–85.
- Marenholz I, Nickel R, Ruschendorf F *et al.* Filaggrin loss-of-function mutations predispose to phenotypes involved in the atopic march. *J Allergy Clin Immunol* 2006; **118**:866–71.
- Morar N, Cookson WO, Harper JL, Moffatt MF. Filaggrin mutations in children with severe atopic dermatitis. *J Invest Dermatol* 2007; **127**:1667–72.
- 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.
- Hudson TJ. Skin barrier function and allergic risk. *Nat Genet* 2006; **38**:399–400.
- Weidinger S, Rodriguez E, Stahl C *et al.* Filaggrin mutations strongly predispose to early-onset and extrinsic atopic dermatitis. *J Invest Dermatol* 2007; **127**:724–6.
- Mancini AJ. Skin. *Pediatrics* 2004; **113**:1114–19.
- Braun-Fahrlander C, Wuthrich B, Gassner M *et al.* Validation of a rhinitis symptom questionnaire (ISAAC core questions) in a population of Swiss school children visiting the school health services. SCARPOL team. Swiss Study on Childhood Allergy and Respiratory Symptom with respect to Air Pollution and Climate. International Study of Asthma and Allergies in Childhood. *Pediatr Allergy Immunol* 1997; **57**:75–82.
- Pearce N, Sunyer J, Cheng S *et al.* Comparison of asthma prevalence in the ISAAC and the ECRHS. *Eur Respir J* 2000; **57**:420–6.
- Hederos CA, Hasselgren M, Hedlin G, Bornehag CG. Comparison of clinically diagnosed asthma with parental assessment of children's asthma in a questionnaire. *Pediatr Allergy Immunol* 2007; **18**:135–41.
- Sandilands A, Smith FJ, Irvine AD, McLean WH. Filaggrin's fuller figure: a glimpse into the genetic architecture of atopic dermatitis. *J Invest Dermatol* 2007; **127**:1282–4.
- Lander ES. The new genomics: global views of biology. *Science* 1996; **274**:536–9.

Supporting Information

Additional Supporting Information may be found in the online version of the article:

Table S1. Candidate single-nucleotide polymorphisms of FLG.

Table S2. Polymerase chain reaction primers used for genotyping of FLG variants.

Please note: Wiley-Blackwell are not responsible for the content or functionality of any supporting materials supplied by the authors. Any queries (other than missing material) should be directed to the corresponding author for the article.

Supplement

Table S1. Candidate SNPs of FLG

Gene	Amino Acid change	PCR Product
FLG	T454A	A
	P478S	
	E498D	
	R501X	
	H519N	
	S3247X	B
	R3270C	
	S3296X	
	Q3322Q	
	L2481S	C
	H2507Q	
	S2554X	
	D2781D	D
	S2889X	
	V3215V	
	2282del4	
	3321delA	
	E1795X	
	E2422X	
	Q2417X	

Table S2. PCR primers used for genotyping of FLG variants

PCR product	Primer-F	Primer-R	PCR Amplicom(bp)
PCR-A	GAACCTGAGTGTCCAGACCTATTTA	CAGAAAACTCAGACACACAATCAGT	346-bp
PCR-B	TGCAGATGAAGCTTGTCCAC	TCTGAGAGGTGGTCTGGGTC	297-bp
PCR-C	CCACACGTGGCCGGTCAGCA	CTGGCTAAA ACTGGATCCCCA	512-bp
PCR-D	GATGGACGGGGCCCAGCACTA	CTTGGTGGCTCTGCTGATGGGA	1108-bp