

# Eczema Genetics: Current State of Knowledge and Future Goals

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Multiple genetic as well as environmental factors interact in the pathogenesis of eczema. Increased understanding of genetic predisposition in atopy and eczema has directed interest toward key pathogenic mechanisms including skin barrier dysfunction. This review provides a succinct update on the current state of knowledge regarding eczema genetics. We discuss the relevance of loss-of-function mutations in the filaggrin gene within the context of other candidate gene studies and suggest possible applications for future research. Knowledge of genetic factors in eczema may translate into a clearer understanding of pathogenic mechanisms and hence more focused therapeutic strategies, but this remains at present a distant possibility.

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## INTRODUCTION

Eczema (Johansson *et al.*, 2004) is a complex trait, that is, multiple genetic and environmental factors contribute to its pathogenesis. There is considerable heterogeneity within the clinical phenotype of “eczema” and the disease is also likely to encompass significant etiological heterogeneity. The subclassification of atopic and nonatopic eczema is not clear-cut (Brown and Reynolds, 2006) and the use of different diagnostic criteria, focusing on different clinical and pathological features (Williams, 2003; Williams and Johansson, 2005), has not facilitated a unified understanding of eczema pathogenesis. However, increasing knowledge of the role of genetic predisposition in atopy and eczema has improved our understanding of this complex trait and directed interest toward key pathogenic mechanisms.

This review aims to provide an update on current understanding of the genetic basis of eczema, focusing on atopic eczema, and to suggest possible future research goals in this fast-moving field.

## EVIDENCE FOR A GENETIC PREDISPOSITION IN ECZEMA

There is substantial evidence in support of a strong genetic component in atopic eczema. Twin studies show concordance rates of 0.72–0.86 in monozygotic compared with 0.21–0.23 in dizygotic twin pairs (Larsen *et al.*, 1986; Schultz Larsen, 1993; Thomsen *et al.*, 2007) and eczema as well as asthma and allergic rhinitis show clustering within families (Kaiser, 2004). Common features of the systemic immune response link eczema with other atopic diseases, however several lines of investigation point to tissue-specific genes, relating to the structure and function of skin, as etiological factors in eczema pathogenesis. Children whose parents have eczema show a higher risk of developing eczema than children whose parents have asthma or hay fever (Moore *et al.*, 2004; Wadonda-Kabondo *et al.*, 2004) and eczema can occur with increased severity along Blaschko's lines (Hladik *et al.*, 2005), consistent with genetic mosaicism (Moss, 1999). These observations support the concept that risk may be

mediated through skin-specific genes, rather than simply through systemic immune or “atopy” risk genes. Recent thinking has focused on the importance of epithelial barrier dysfunction in atopic eczema, directed by evidence from genetics as well as biochemical and physiological studies (Cookson, 2004; Hudson, 2006; Irvine and McLean, 2006; Jakasa *et al.*, 2006, 2007; Taieb *et al.*, 2006).

## STRATEGIES FOR INVESTIGATING THE GENETIC BASIS OF ATOPIC ECZEMA

A large amount of research has been undertaken worldwide in the search for genetic factors in the etiology of atopy and eczema. Three main approaches have been used: candidate gene association, selecting genes for study based on a hypothesis of known biological function; genome-wide linkage screens, which are hypothesis free and compare the transmission of genetic information between cases and controls in family pedigrees; and DNA microarray studies, which look at gene expression in selected regions of inter-

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Abbreviations: FLG, filaggrin; SPINK5, serine protease inhibitor Kazal type 5; UCA, urocanic acid

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est, or across the whole genome. Each of these strategies has added understanding to the field of eczema genetics.

It is of paramount importance to replicate previously reported findings using different methodology, independent population groups, and larger sample sizes, to confirm a robust and clinically relevant association (Hoffjan and Epplen, 2005; Morar *et al.*, 2006b). Many striking preliminary discoveries have failed to be replicated in subsequent studies.

### POINTERS FROM GENOME-WIDE STUDIES

Genome-wide linkage screens have been reported in families with atopic eczema from five different populations (Lee *et al.*, 2000; Cookson *et al.*, 2001; Bradley *et al.*, 2002; Haagerup *et al.*, 2004; Enomoto *et al.*, 2007). Regions on chromosomes 3q, 3p, 17q, and 18q show evidence of linkage in two or more studies, suggesting candidate sites for eczema genes (Hoffjan and Epplen, 2005). Interestingly, only two of these four regions have shown linkage to asthma or other atopic disorders in more than one study (Cookson, 2004; Hoffjan and Epplen, 2005) indicating that separate genes may be responsible for eczema. Furthermore, the regions on 1q21, 3q21, 17q25, and 20p linked to atopic eczema overlap with known psoriasis susceptibility loci (Cookson, 2004) although a subsequent investigation of the 17q25 locus failed to demonstrate variants in the known PSORS2 psoriasis locus in children with atopic eczema (Morar *et al.*, 2006a). The colocalization of eczema and psoriasis genes supports the concept that tissue-specific genes influence the eczema phenotype by control of epidermal function, immunity, and inflammation (Cookson, 2004). Notably, the 1q21 locus includes the epidermal differentiation complex, a dense cluster of genes including filaggrin (*FLG*), involucrin, loricrin and S100 proteins, which are involved in the terminal differentiation of keratinocytes (Mischke *et al.*, 1996).

A large-scale DNA microarray analysis has demonstrated that four genes encoded on 1q21 (overlying

the epidermal differentiation complex) show different levels of expression in eczema lesional skin compared with controls (Sugiura *et al.*, 2005). These findings suggest that S100A7 and S100A8 (showing increased expression) plus filaggrin and loricrin (showing reduced expression) may be responsible at least in part for the epidermal barrier dysfunction that is characteristic of atopic eczema. Another independent microarray analysis has shown an increase in the expression of genes encoding CC chemokines in eczematous skin (Nomura *et al.*, 2007), known to attract T<sub>H</sub>2 cells and eosinophils. The functional significance of these plus other genes and partial DNA sequences identified by DNA microarray analysis remain to be elucidated (Saito, 2005).

### CANDIDATE GENES FOR ECZEMA AND ATOPY

Many different candidate genes have been studied because of their theoretical roles in the etiology of atopic eczema; most candidate studies have focused on immunological mechanisms and several genes known to be associated with asthma/atopy have been investigated for linkage with atopic eczema. At least 20 genes have been reported as showing a statistically significant association with eczema, but only six associations have been replicated in two or more independent studies (Maintz and Novak, 2007; Kiyohara *et al.*, 2008). These findings are summarized in Figure 1.

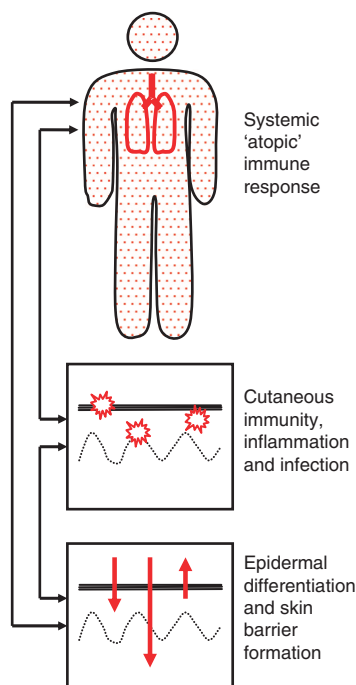
Three genes within the 5q31–33 locus show evidence of association with atopic eczema: the cytokines IL-4 and IL-13 and a protease inhibitor, SPINK5 (serine protease inhibitor Kazal type 5). Identification of mutations in the *SPINK5* gene as the cause of Netherton syndrome (Chavanas *et al.*, 2000) led to the screening of this gene for polymorphisms in patients with atopic eczema, as Netherton syndrome is characterized by an eczematous rash and elevated IgE. *SPINK5* encodes lymphoepithelial Kazal type inhibitor that is expressed in epithelial surfaces (Komatsu *et al.*, 2002) and it has been suggested that lymphoepithelial Kazal type inhibitor may provide protection

against other serine proteases that are allergenic (Walley *et al.*, 2001). Polymorphisms in *SPINK5* have shown association with atopic eczema in cohorts from Britain and Japan (Walley *et al.*, 2001; Kato *et al.*, 2003; Nishio *et al.*, 2003), but this association has not been replicated in subsequent studies (Folster-Holst *et al.*, 2005), with the exception of a minor parent-of-origin effect when maternally inherited (Weidinger *et al.*, 2008a).

The stratum corneum chymotryptic enzyme is involved in the proteolytic degradation of corneodesmosomes, regulating desquamation from the skin surface (Ekholm *et al.*, 2000). The stratum corneum chymotryptic enzyme gene has been reported as a candidate in atopic eczema (Vasilopoulos *et al.*, 2004) but again this association has not been replicated in our larger cohort study (Weidinger *et al.*, 2008a).

There are many other candidate genes that showed interesting associations that have failed to be replicated by subsequent studies. Examples include the high-affinity IgE receptor (FcεR1-β), which showed linkage to atopy but inconsistent findings in eczema (Coleman *et al.*, 1993; Sandford *et al.*, 1993; Soderhall *et al.*, 2001); nucleotide-binding oligomerization domain proteins 1 and 2, which are intracellular receptors for bacterial peptides (Kabesch *et al.*, 2003; Weidinger *et al.*, 2005a,b); and toll-like receptor 2, which is expressed on human keratinocytes (McInturff *et al.*, 2005) and recognizes pathogen-associated molecular patterns (Ahmad-Nejad *et al.*, 2004; Weidinger *et al.*, 2006b; Merx *et al.*, 2007). These conflicting reports and lack of replication may result from inadequate statistical power or suboptimal study design. Careful case-definition and choice of an appropriate, comparable control population are important considerations in genetic association studies and large sample sizes are necessary to detect or refute subtle but potentially important genetic effects (Balding, 2006).

In contrast to the lack of replication shown by many candidate genes, *FLG* has now been reported to show a strong and significant association



| Gene showing association with atopic eczema              | Locus        | Rationale                                                                                              | References                                                                                                                 |
|----------------------------------------------------------|--------------|--------------------------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------|
| Interleukin-4 ( <i>IL4</i> )                             | 5q31–33      | Interleukin 4 promotes the switch to $T_H2$ immune response and IgE production                         | (He <i>et al.</i> , 2003; Kawashima <i>et al.</i> , 1998)                                                                  |
| Interleukin-4 receptor ( <i>IL4R</i> )                   | 16p12.1–11.2 |                                                                                                        | (He <i>et al.</i> , 2003; Hosomi <i>et al.</i> , 2004; Oiso <i>et al.</i> , 2000)                                          |
| Interleukin-13 ( <i>IL13</i> )                           | 5q31–33      | Interleukin 13 promotes B-cell switch to IgE production                                                | (Liu <i>et al.</i> , 2000; Tsunemi <i>et al.</i> , 2002)                                                                   |
| Mast cell chymase ( <i>CMA1</i> )                        | 14q11.2      | Mast cell chymase promotes increased microvascular permeability and accumulation of inflammatory cells | (Mao <i>et al.</i> , 1996; Weidinger <i>et al.</i> , 2005)                                                                 |
| Serine protease inhibitor kazal-type 5 ( <i>SPINK5</i> ) | 5q31         | Mutations in <i>SPINK5</i> gene cause netherton syndrome                                               | (Kato <i>et al.</i> , 2003; Nishio <i>et al.</i> , 2003; Walley <i>et al.</i> , 2001)                                      |
| Filaggrin ( <i>FLG</i> )                                 | 1q21         | <i>FLG</i> null mutations cause ichthyosis vulgaris                                                    | (Palmer <i>et al.</i> , 2006) plus >15 other reports reviewed in: (Brown and Irvine, 2008; Rodriguez <i>et al.</i> , 2008) |

**Figure 1. Diagram summarising genes reported to show association with atopic eczema in two or more independent studies.** The majority of research into genetic causes of atopy and eczema originally focused on the systemic immune response, which characteristically shows a  $T_H2$  predominance and elevated IgE levels. However, eczema and asthma do not appear to share the same genetic loci (Cookson, 2004; Hoffjan and Epplen, 2005; Veal *et al.*, 2005) and more recently interest has focused on skin-specific genes involved in cutaneous immunity, inflammation, and infection as well as epidermal barrier function. Although genes may be categorized according to their primary functional site, there is clearly significant overlap and interplay between systemic and cutaneous immunity (both innate and acquired) and the epidermal barrier that has a key function in limiting the entry of allergens and infectious agents.

with atopic eczema in more than 15 separate publications, many of which contain a number of additional within-study replications (Brown and Irvine, 2008; Rodriguez *et al.*, 2008).

### FILAGGRIN WITHIN THE EPIDERMIS: A MULTIFUNCTIONAL PROTEIN

Filaggrin (filament-aggregating protein) has a key function in epidermal differentiation and barrier function, but the relative importance of the different functions of filaggrin remains unclear (Figure 2). The gene *FLG* encodes a large insoluble polypeptide, profilaggrin (Sybert *et al.*, 1985), which is cleaved to produce multiple filaggrin peptides (Gan *et al.*, 1990). Filaggrin aggregates keratin 1, keratin 10 and other intermediate filaments within the cytoskeleton of keratinocytes (Manabe *et al.*, 1991), to bring about their compaction during cornification, a unique form of programmed cell

death (Candi *et al.*, 2005). Filaggrin is a major component of the protein-lipid cornified cell envelope, which forms an important permeability barrier to water and inhibits the entry of microbes, allergens, and irritants (Presland *et al.*, 2004).

Filaggrin is degraded to produce a mixture of hygroscopic amino acids that may contribute to epidermal barrier function by retaining water and increasing flexibility of the cornified layer (Rawlings and Harding, 2004). One of the most abundant amino acids released by filaggrin proteolysis is histidine. This is converted to *trans*-urocanic acid (*trans*-UCA) and helps to maintain the pH gradient of the epidermis. The *trans*-UCA molecule undergoes photoisomerization to *cis*-UCA (Elias and Choi, 2005), which has local and systemic immunosuppressive effects, demonstrated in murine models (Noonan and De Fabo, 1992; Finlay-Jones and Hart, 1998; Walterscheid

*et al.*, 2006) and in human keratinocytes and leukocytes *in vitro* (Gilmour *et al.*, 1993; McLoone *et al.*, 2005). Finally, the N-terminal portion (S100 domain) of profilaggrin is a calcium-binding domain (Presland *et al.*, 1992, 1995; Markova *et al.*, 1993) and may therefore be involved in the regulation of calcium-dependent events during epidermal differentiation (Presland *et al.*, 1997).

### FLG, THE STRONGEST CANDIDATE SO FAR

In 2006, a group led by Prof Irwin McLean (University of Dundee) reported that two common loss-of-function mutations within the *FLG* gene cause ichthyosis vulgaris (Smith *et al.*, 2006). The coexistence of ichthyosis vulgaris and atopic eczema is well recognized in clinical practice and investigation of the 15 families with ichthyosis vulgaris demonstrated a significant association between atopic

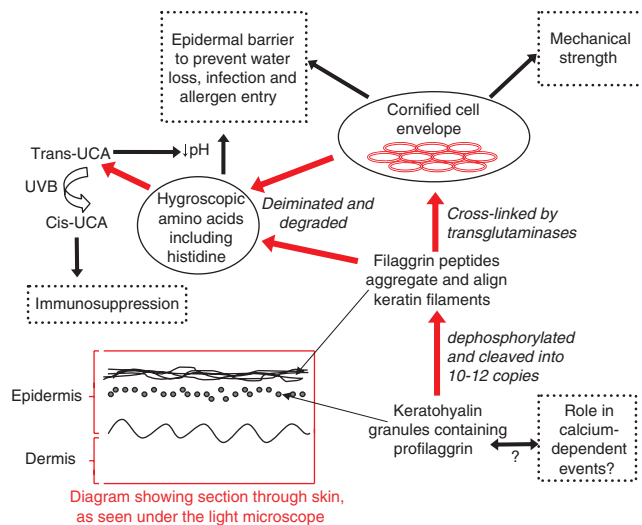


**Figure 2. Diagrammatic representation of the processing and functions of filaggrin within the epidermis.** Profilaggrin is expressed in the granular layer and is the major constituent of keratohyalin granules that are clearly visible under the light microscope. Profilaggrin is proteolytically cleaved in the uppermost granular cells to produce filaggrin peptides that aggregate and align keratin filaments within the cytoskeleton of keratinocytes, helping to bring about their compaction in to a squame shape during cornification (Candi *et al.*, 2005). The protein-lipid cornified cell envelope, which replaces the keratinocyte cell membrane, forms an important permeability barrier and provides mechanical defense by maintaining skin integrity (Presland *et al.*, 2004). Filaggrin is deiminated and degraded with a half-life of 6 hours (Candi *et al.*, 2005), to release hygroscopic amino acids. These may contribute to epidermal barrier function by retaining water and are also thought to help maintain the pH gradient in healthy skin. There is some experimental evidence that histidine, after metabolism to urocanic acid, may be involved in UV light-induced immunosuppression (Gilmour *et al.* 1993; McLoone *et al.* 2005; Walterscheid *et al.* 2006). Finally, profilaggrin includes a calcium-binding domain and may therefore be involved in signal transduction (Markova *et al.*, 1993). *Trans*-UCA, *trans*-urocanic acid, produced by non-oxidative deimination of histidine; *cis*-UCA, *cis*-urocanic acid produced by photoisomerization; UVB, UV B radiation. This figure is modified from Brown and Irvine (2008) with permission from Elsevier.

dermatitis and the two *FLG* null alleles, R501X and 2282del4, with an estimated LOD score (LOD to the base 10) of 3.08–3.27 (Palmer *et al.*, 2006). An LOD score of  $\geq 3$  is considered to indicate significant linkage and for a complex trait this was a striking finding. The group then proceeded to study three additional eczema cohorts and control populations from other sources in Ireland, Scotland, and Denmark, replicating the finding and showing a strong and highly significant association between *FLG* null mutations and atopic eczema, with estimated odds ratios of up to 13.4 (95% confidence interval 6.2–27.5), and  $\chi^2$ -test  $P = 3 \times 10^{-17}$  (Palmer *et al.*, 2006).

More than 20 loss-of-function mutations within the *FLG* gene have now been reported (Palmer *et al.*, 2006; Sandilands *et al.*, 2006; Chen *et al.*, 2008; Nomura *et al.*, 2008; Sasaki *et al.*, 2008; Figure 3). Five null mutations are prevalent within the

European population, giving a combined null allele frequency of 0.09, meaning that about 9% of the well-studied Irish population, for example, carry one or more *FLG* null mutations. This strikingly high prevalence suggests a possible heterozygote advantage. In this regard, a form of “natural vaccination” mediated by the mildly perturbed skin barrier in heterozygotes has been hypothesized (Sandilands *et al.*, 2007a,b), so that atopy may be a modern plague that is enriched among the survivors of ancient bacterial plagues (Irvine and McLean, 2006). The other rarer mutations within the European population were identified in families with ichthyosis vulgaris and some may be family specific (Sandilands *et al.*, 2007a,b). Population-specific mutations have also been identified in association with ichthyosis vulgaris and atopic eczema in Japanese (Nomura *et al.*, 2007, 2008; Enomoto *et al.*, 2008; Hamada *et al.*, 2008) and

Singaporean Chinese populations (Chen *et al.*, 2008).

All the reported *FLG* mutations are nonsense or frameshift mutations, each resulting in truncation of the profilaggrin molecule. However, biochemical and immunohistochemical studies indicate that the truncated profilaggrin cannot be processed into filaggrin peptides (Sandilands *et al.*, 2006, 2007a). Hence, even mutations occurring near the 3' end of *FLG* result in a similar reduction or complete absence of filaggrin as well as a similarly severe phenotype and with a statistically similar effect (Brown *et al.*, 2008c). This equivalence of effect explains the rationale for statistical analysis using a “combined null genotype” that has been applied in most studies. This technique groups together individuals with any one or any combination of two of the known *FLG* null mutations, rather than analyzing each mutation separately.

Association of the *FLG* null genotype with atopic eczema has now been replicated in numerous case-control studies (Marenholz *et al.*, 2006; Palmer *et al.*, 2006; Ruether *et al.*, 2006; Barker *et al.*, 2007; Stemmler *et al.*, 2007; Weidinger *et al.*, 2007; Sandilands *et al.*, 2007b; Novak *et al.*, 2008; Brown *et al.*, 2008c), as well as family studies (Weidinger *et al.*, 2006a; Morar *et al.*, 2007; Ekelund *et al.*, 2008) and most recently population-based studies (Henderson *et al.*, 2008; Brown *et al.*, 2008b; Weidinger *et al.*, 2008b). Some publications were in collaboration with the original research group; others have independently replicated and extended the data. There has to date been only one published study that did not show an association between *FLG* null mutations and atopic eczema (Giardina *et al.*, 2008). In this Italian population, the R501X and 2282del4 mutations occurred at a very low frequency and screening for other reported mutations or previously unreported Italian mutations was not performed.

Given that the association of *FLG* with eczema is one of the most robust and highly significant discoveries in the field of complex trait genetics, it is reasonable to ask why *FLG* was not detected by whole-genome linkage

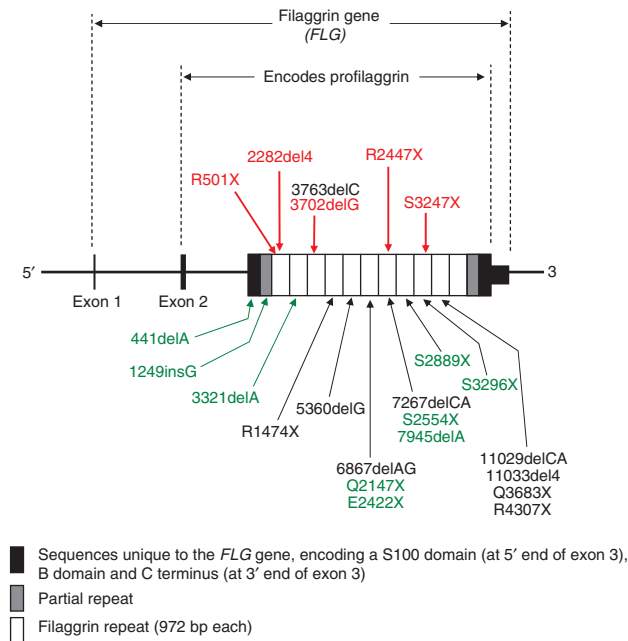


**Figure 3. Diagrammatic representation of the filaggrin (*FLG*) gene showing sites of reported null mutations.** Diagram adapted from Sandilands *et al.* (2007a, b) and Brown and Irvine (2008) with additional recently reported mutations in non-white populations (Chen *et al.*, 2008; Nomura *et al.*, 2008; Sasaki *et al.*, 2008). *FLG* is located on chromosome 1q21, within the epidermal differentiation complex, a cluster of genes controlling the terminal differentiation of keratinocytes. Exons 1 and 2 showed no variation between individuals with and without ichthyosis vulgaris in five families (Smith *et al.*, 2006). Exon 2 contains the initiation codon; exon 3 is unusually large and encodes most of the N-terminal domain as well as all of the *FLG* repeats and their linker sequences. Profilaggrin is polymorphic in that there are at least four alleles in the human population encoding 10, 11, or 12 *FLG* repeats (Gan *et al.*, 1990; Sandilands *et al.*, 2007a, b). The mutations marked in red are prevalent in the European population, with a combined allele frequency of 0.09. The mutations marked in black are found in European and European American populations at much lower frequencies. The mutations marked in green have been identified in ichthyosis vulgaris and eczema cases from Chinese, Japanese, and Singaporean Chinese populations.

The odds ratio for this milder phenotype is around 2.0, similar to the odds ratio seen in family studies (Baurecht *et al.*, 2007), presumably reflecting milder disease. In one population study, only those children who were homozygous for *FLG* null mutations showed a statistically significant association with mild-moderate atopic eczema (odds ratio 26.9, 95% confidence interval 3.3–217.1), whereas the *FLG* null heterozygotes did not have a significantly increased risk of eczema (odds ratio 1.2, 95% confidence interval 0.7–1.9) suggesting a “recessive” type of inheritance (Brown *et al.*, 2008b). In ichthyosis vulgaris, the inheritance is semidominant (that is, the heterozygotes demonstrate a milder phenotype than the homozygote null mutants) (Smith *et al.*, 2006) and in moderate-severe eczema cases both the *FLG* null heterozygotes and null homozygotes show an increased risk of disease (Palmer *et al.*, 2006; Brown *et al.*, 2008c). The different patterns of inheritance may perhaps be explained by the coinheritance of genetic and environmental risk factors in the more severe disease phenotype.

Careful dermatological examination can be used to help identify individuals carrying one or more *FLG* null alleles. Eczema in the context of ichthyosis vulgaris is clearly likely to be related to filaggrin deficiency. However the clinical signs of palmar hyperlinearity (Weidinger *et al.*, 2006a; Novak *et al.*, 2008; Brown *et al.*, 2008b) and keratosis pilaris (Brown *et al.*, 2008b) have also each shown independent association with *FLG* null mutations; furthermore, the absence of both palmar hyperlinearity and keratosis pilaris gives a negative predictive value of 92% (that is, 92% of children without these features do not carry a *FLG* null mutation) (Brown *et al.*, 2008a).

The compound phenotype of asthma in the context of atopic eczema has also shown a striking association with *FLG* null mutations, with estimated odds ratios as high as 5.69 (95% confidence interval 2.76–11.37) (Weidinger *et al.*, 2007). The association of *FLG* with other atopic phenotypes is reviewed elsewhere (Rodriguez *et al.*, 2008).

analysis? The 1q21 locus, which includes the epidermal differentiation complex, was detected as a significant linkage peak on one whole-genome screen (Cookson *et al.*, 2001). However, the whole-genome analyses reported to date have each used low-density microsatellite markers at insufficient density to detect the multiple different *FLG* null mutations. The genetic architecture of *FLG* is highly complex; there are many low-frequency null variants, each of which will be poorly, or at best incompletely, tagged by conventional single nucleotide polymorphisms used in genome-wide analysis, as the latter are deliberately selected on the basis of high heterozygote frequencies in the population (Sandilands *et al.*, 2007a, b). Variations in case definition and use of non-homogeneous population struc-

ture, that is, ethnically mixed study populations, may also have been confounding factors.

### GENOTYPE-PHENOTYPE CORRELATION

The phenotype of early onset (before the age of 2 years), persistent, and severe eczema has shown the strongest and most highly significant statistical association with the combined *FLG* null genotype, having an odds ratio of up to 7.7 (95% confidence interval 5.3–10.9) (Barker *et al.*, 2007; Weidinger *et al.*, 2007; Brown *et al.*, 2008c). However, the milder eczema phenotype that is more prevalent in the general population has also shown a statistically significant association with *FLG* null genotype in population studies (Henderson *et al.*, 2008; Brown *et al.*, 2008b; Weidinger *et al.*, 2008b).

## GENETIC STUDIES IN OTHER TYPES OF DERMATITIS

Genetic factors contributing to the pathogenesis of nonatopic eczema and the other forms of dermatitis (Johansson *et al.*, 2004) have not been extensively investigated and are not clearly defined.

Environmental factors obviously have an important function in the development and perpetuation of allergic as well as irritant contact dermatitis. However, an individual variability in susceptibility to allergic contact dermatitis has long been recognized and genetic factors are likely to be involved in mediating this susceptibility (Schram and Warshaw, 2007). Definition of a precise phenotype of contact allergy for the purposes of genetic research is difficult. It has therefore been proposed that polysensitization to contact allergens (defined as sensitization to three or more unrelated allergens) is a useful phenotypic subgroup in which to study genetic polymorphisms relating to contact allergy-specific as well as nonspecific inflammatory processes (Schnuch *et al.*, 2008). These authors have reported polymorphisms in the promoter regions of IL-16 and tumor necrosis factor- $\alpha$  in association with the polysensitized phenotype (Reich *et al.*, 2003; Westphal *et al.*, 2003). In contrast, specific contact allergies may be related to specific pathogenic mechanisms; examples include acetylation in *para*-substituted arylamine-induced contact allergy, associated with polymorphisms in genes encoding some *N*-acetyltransferases (Westphal *et al.*, 2000) and the unique features of nickel as a contact allergen (Schram and Warshaw, 2007; Hanifin, 2008).

A polymorphism in the tumor necrosis factor- $\alpha$  gene at position 308 has shown association with susceptibility to irritant contact dermatitis (Allen *et al.*, 2000). However, a more recent study of cytokine gene polymorphisms and irritant hand dermatitis found no difference in genotype distributions between cases and controls, although subgroup analysis did suggest a possibly increased susceptibility in individuals with the tumor necrosis factor- $\alpha$ -308 polymorphism and a possible protective effect of an IL-1 $\alpha$  polymorphism (de Jongh *et al.*, 2008a).

Theoretically, an impaired epidermal barrier may permit the entry of allergens and irritants. However, the significance of filaggrin deficiency in allergic and/or irritant contact dermatitis in the absence of atopy currently remains unclear (Lerbaek *et al.*, 2007; Brown and Cordell, 2008; Hanifin, 2008; Novak *et al.*, 2008; de Jongh *et al.*, 2008b).

## FILAGGRIN IN CONTEXT

The challenge now is to place our understanding of the importance of *FLG* null mutations in an appropriate context within the pathogenesis of eczema and atopy. Clearly, filaggrin haploinsufficiency is an important and significant component in the pathogenesis of a proportion of eczema cases but it cannot be used to explain the majority of eczema, even in most of the severe case series. Therefore, looking objectively at the published data, what is the significance of *FLG* in eczema?

Meta-analysis of nine studies has estimated an odds ratio of 4.09 (95% confidence interval 2.64–6.33) for atopic eczema from case-control studies and 2.06 (95% confidence interval 1.76–2.42) from family studies, for the combined null genotype of R501X and 2282del4 (Baurecht *et al.*, 2007). Other candidate genes showing somewhat comparable odds ratios include *IL-4*, odds ratio 1.88,  $P=0.01$  (Kawashima *et al.*, 1998); *IL-13*, odds ratio 1.7,  $P=0.03$  (Liu *et al.*, 2000) and relative risk 2.5,  $P=0.014$  (He *et al.*, 2003); and mast cell chymase odds ratio 2.17,  $P=0.009$  (Mao *et al.*, 1996). These significant associations have been replicated in two or more studies and should therefore feature alongside *FLG* in our understanding of genetic predisposition to eczema. Individuals carrying mutations in more than one candidate gene may, theoretically, demonstrate an additive or multiplicative effect; this possibility has been investigated in one study, but significant epistatic effects between *FLG*, *SPINK5*, and *KLK7* were not identified (Weidinger *et al.*, 2008a).

In the moderate-severe eczema case series, 45.7–56% of cases carry one or more *FLG* null mutations (Palmer *et al.*, 2006; Barker *et al.*, 2007; Brown *et al.*,

2008c). However, the population attributable risk fraction has been variably estimated at between 4.2 and 15.1% in English and German populations (Marenholz *et al.*, 2006; Henderson *et al.*, 2008; Brown *et al.*, 2008b; Weidinger *et al.*, 2008b). Hence, approximately 50% of moderate-severe eczema cases may be attributed at least in part to *FLG* null mutations, whereas only up to 15% of mild-moderate eczema may be explained by *FLG* on a population scale. Population-level statistics are not currently available for other candidate genes in atopic eczema.

Can we use this understanding of *FLG* in eczema to help identify other factors contributing to the pathogenesis of this heterogeneous disease? Barrier dysfunction is a characteristic feature of eczema in lesional and non-lesional skin (Akdis *et al.*, 2006; Jakasa *et al.*, 2006, 2007) and filaggrin deficiency as a result of *FLG* null mutations appears to contribute to this feature (Kezic *et al.*, 2008). Examples of other genes, as well as gene-gene and gene-environment interactions contributing to barrier dysfunction have been demonstrated but require further investigation. Caspase 14, a protease involved in keratinocyte terminal differentiation, may contribute to epidermal barrier dysfunction through mechanisms including filaggrin insufficiency (Denecker *et al.*, 2008); *FLG* expression is down-regulated in acute eczematous skin and in keratinocytes cultured with IL-4 and IL-13 (Howell *et al.*, 2007) demonstrating the interplay with immunity and inflammation; expression of profilaggrin in human skin is reduced after experimental barrier disruption (Torma *et al.*, 2007); and *FLG* null mutations in combination with cat ownership at birth may predispose to infantile eczema (Bisgaard *et al.*, 2008), showing a possible role for barrier dysfunction in allergy, although surprisingly, the association was independent of sensitization to cats (assessed by skin-prick testing). A subclassification of eczema cases into *FLG*-associated and *FLG*-independent disease may therefore be useful to facilitate the identification of other genetic factors and pathogenic mechanisms. Similarly, the role of

environmental factors may be more apparent and amenable to study in eczema cases having controlled for the presence/absence of *FLG* null mutations.

## FUTURE GOALS

The most urgent and attractive goal is to translate our knowledge of genetic predisposition into a greater understanding of molecular mechanisms in the pathogenesis of eczema, to guide the development of previously unreported therapies for this common and distressing disease. To date, identification of the candidate genes discussed above has not been translated into therapeutic advances for eczema patients. Filaggrin haploinsufficiency has theoretical potential as a therapeutic target. The wild-type allele carried by a heterozygote might be upregulated by small molecules acting on pathways controlling *FLG* gene expression. In homozygous or compound heterozygous carriers of null mutations, molecules that enhance the cell's translational machinery to read through premature termination codon mutations (Ainsworth, 2005) are a possible therapeutic avenue. The search for both these classes of small molecules is well underway in the McLean laboratory.

The rational design of novel therapeutic interventions would be facilitated by a clearer understanding of the mechanisms by which filaggrin, its precursor, protein and breakdown products each exert their effects in healthy skin and eczema (Figure 2). It is not known exactly how filaggrin interacts with keratin filaments on a molecular level, to bring about the orderly collapse of keratinocytes in the stratum corneum. The contribution of amino acids released by filaggrin degradation to epidermal barrier function is unclear and quantitative analysis of barrier function may clarify this effect. Similarly, the proposed role of histidine in UV light-induced cutaneous immunosuppression requires clarification. There are some limited data to suggest that filaggrin repeat number polymorphisms (the variable number of filaggrin subunits repeated within the profilaggrin molecule) may be correlated with dry skin phenotype

(Ginger *et al.*, 2005) but this study was performed before the discovery of *FLG* null mutations so the data are difficult to interpret. It could be that copy number variation (Redon *et al.*, 2006) within and around the *FLG* gene locus also contributes to eczema susceptibility and it is theoretically possible that this dose effect may even be of a similar significance to the null mutations themselves. Calcium is important in the intracellular signaling pathways of epidermal differentiation and inflammation. However, the function of profilaggrin as a calcium-binding molecule for signal transduction has not been studied in detail.

Of relevance to clinical practice, individuals with filaggrin insufficiency are at increased risk of severe, persistent eczema and future therapy may place greater emphasis on barrier repair for this subgroup of patients. Eczema is a disease in which pharmacogenetics may facilitate the development of primary prevention and treatment regimes tailored according to the individual's genetic predisposition.

The technical ability to perform more detailed, large-scale, and high-density genome-wide association analyses has recently improved understanding of the genetic basis of other complex traits, including inflammatory bowel disease, diabetes, and breast cancer (Wellcome Trust Case Control Consortium, 2007). Previously unreported susceptibility loci have been identified, offering new insight into pathogenic mechanisms in these diseases (McCarthy *et al.*, 2008); the results of genome-wide association studies for atopic eczema are awaited with interest. However, current technology is still only able to assess a small proportion of potential genetic variation (Redon *et al.*, 2006; Stranger *et al.*, 2007), so a complete understanding of individual genetic susceptibility remains a distant possibility (McCarthy *et al.*, 2008; Pattin and Moore, 2008). Large, well-designed studies with careful definition of cases, appropriate control populations, and scrupulous attention to detail throughout the genotyping process (McCarthy *et al.*, 2008) will be required to clarify the roles of genetic and environmental factors in

eczema pathogenesis. Furthermore, the enormous amount of data generated by such comprehensive studies necessitate rigorous analysis, with careful application of appropriate statistical techniques (Cordell and Clayton, 2005; Balding, 2006).

In conclusion, the discovery of *FLG* null mutations has been a major breakthrough in understanding genetic susceptibility to atopy and eczema. However, a very large amount of work is still required to understand fully the multiple interrelated genetic factors contributing to eczema pathogenesis. Furthermore, the potential translation of this understanding for the benefit of patient care represents a considerable challenge for the future.

## CONFLICT OF INTEREST

Irwin McLean has filed patents relating to genetic testing and therapy development aimed at the *FLG* gene.

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