

Filaggrin mutations and atopy: consequences for future therapeutics

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Filaggrin is a key component of the epidermal differentiation complex of the stratum corneum in the epidermal layer of human skin. Loss-of-function mutations in filaggrin have been described in patients with atopic eczema and are associated with an increased risk of atopic sensitization in these individuals. Atopic eczema is the first stage of the atopic march that describes the phenomenon of increased rates of allergic rhinitis and allergic asthma observed in individuals with early atopic dermatitis. The skin barrier disruptions of atopic eczema associated with loss-of-function mutations in filaggrin are thought to provide a nidus for allergic sensitization to food and aeroallergens, which can then lead to increased allergic disease. It is on this foundation that therapies aimed at restoration of barrier function are thought to play a role, not only in the effective treatment of atopic eczema, but also in the prevention of further allergic disease development.

KEYWORDS: atopic march • atopy • epidermal differentiation complex • filaggrin • TSLP

Filaggrin

Filaggrin is a S100 calcium-binding protein with key roles in establishing the skin's epidermal barrier function. It is expressed in the skin and in the nose up to the transitional epithelium as well as the keratinized epithelium of the conjunctiva and oral cavity. Filaggrin is not found on deeper mucosal surfaces of the nose, lung or gut. Its gene is encoded on chromosome 1q21 as part of the epidermal differentiation complex (EDC). The EDC is a cluster of genes crucial to formation of the stratum corneum of the epidermis. Filaggrin is secreted as a phosphorylated polypeptide (profilaggrin), which is predominantly found in the granular cell layer of the epidermis. Profilaggrin consists of 10–12 tandem repeats of filaggrin units. In the granular layer as calcium concentrations increase, profilaggrin is cleaved and dephosphorylated by serine proteases to release functional filaggrin. Filaggrin exists for 6 h prior to full proteolysis (FIGURE 1) [1,2].

Functional filaggrin binds to keratin filaments as the cells become the lipid bilayer and contributes to keratin filament compaction and barrier integrity at the outer layer of the skin known as the cornified envelope. As the cornified envelope forms, filaggrin ensures appropriate barrier function by facilitating collapse of the keratinocyte cytoskeleton to create a dense collection

of proteins and lipids that are crosslinked by transglutaminases. Filaggrin is later modified by caspase 14 to produce hydrophilic acids including alanine, urocanic acid and pyrrolidone carboxylic acid. These acids are a significant component of natural moisturizing factor, which is a key contributor to maintenance of hydration of the outermost layer of the skin known as the stratum corneum. In addition, these acids are significant contributors to maintaining a slightly acidic normal cutaneous pH of approximately 6. Natural moisturizing factor is thought to play a crucial role in maintaining barrier integrity and skin antimicrobial defense (FIGURE 2) [1,3].

Filaggrin in skin disorders

Ichthyosis vulgaris, a skin condition of disordered skin keratinization and scaling, has been linked to loss of function filaggrin mutations inherited in a semidominant fashion [4]. The first identified filaggrin mutations were R501X and 2282del4, but there are now 23 described mutations in patients of European descent, and 30 described mutations in patients of Asian descent [5,6]. In addition, there was no predominant mutation identified in a recent large cohort of Asian patients, although only the C3321delA mutation was seen across patient populations from China, Singapore, Japan, Korea and Taiwan. By contrast,

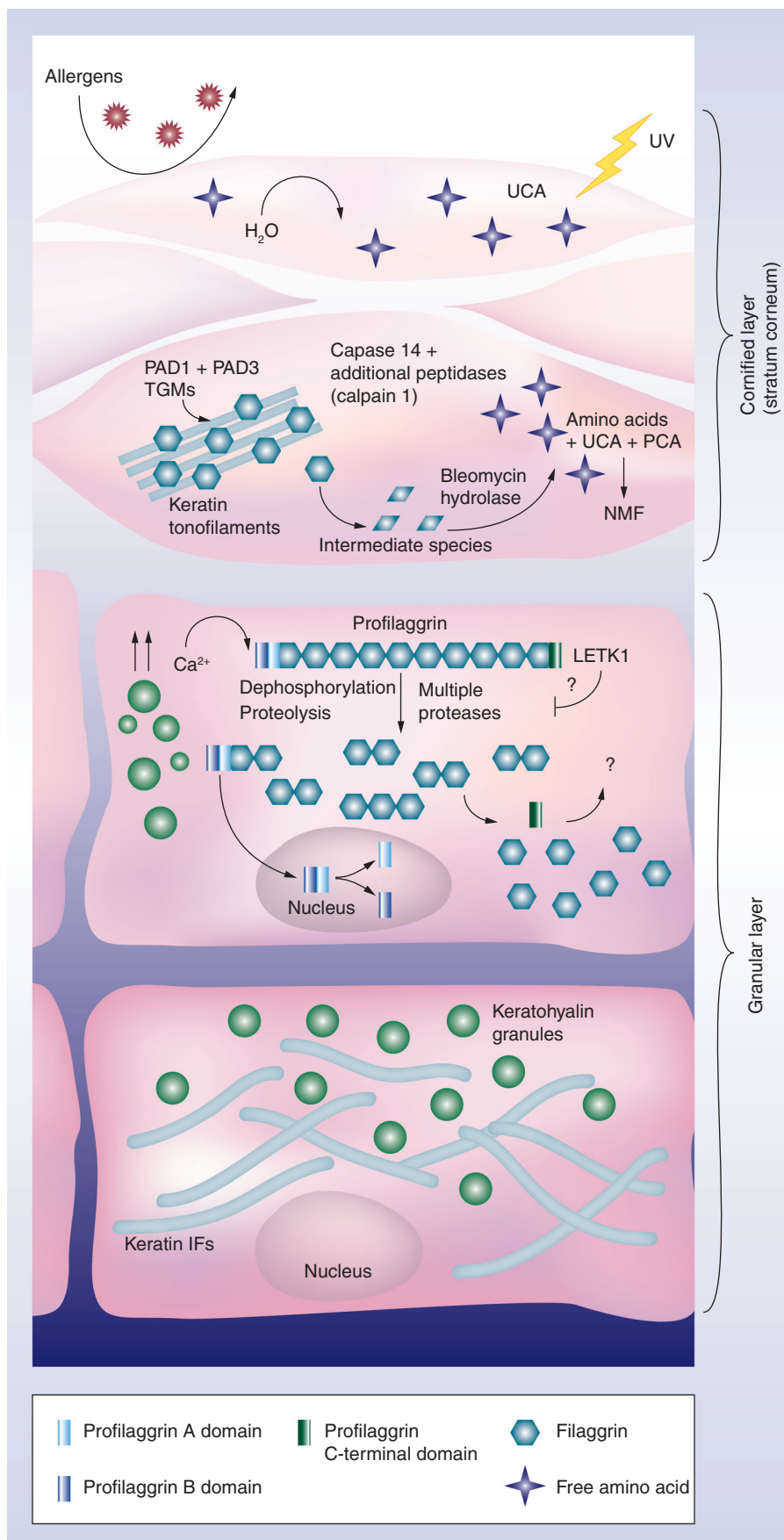


Figure 1. Profilaggrin processing during terminal differentiation of the epidermis. Within the granular layer, profilaggrin is stored in an inactive and insoluble form within keratohyalin granules. In response to an increase in Ca²⁺ levels, the keratohyalin granules degranulate and profilaggrin is dephosphorylated and proteolysed in a multistep process into free filaggrin monomers by a variety of proteases including matriptase, prostasin and probably kallikrein 5 (see text for details). Following cleavage from the filaggrin monomers, the N-terminal head domain undergoes nuclear translocation and further degradation into the A and B domains. In the cornified layer (stratum corneum), the released filaggrin monomers bind directly to keratin filaments, causing their collapse into thickened and aggregated keratin filaments, which has the effect of condensing the keratinocyte cytoskeleton. Condensation of the cytoskeleton is followed by crosslinking with transglutaminases and modification by PADs to form an insoluble keratin matrix. Together with lipids and other cornified-layer proteins, this ultimately forms the so-called 'skin barrier', which prevents water loss through the skin as well as the unwanted entry of molecules such as allergens. Filaggrin undergoes subsequent degradation by a variety of proteases, including caspase 14, into free amino acids and derivatives such as UCA and PCA – these are collectively referred to as NMF, which contributes to skin hydration and possibly to UV protection.

IF: Intermediate filament; NMF: Natural moisturising factor; PAD: Peptidylarginine deiminase; UCA: Uroic acid; TGM: Transglutaminase.

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80% of European patients with filaggrin mutations carry the hotspot mutations R501X or 2282del4. In patients of African descent, the R501X and 2282del4 mutations are uncommon, but have been described in these patients. There is a carrier frequency of just under 10% for filaggrin mutations in European populations [7]. Filaggrin null mutations have incomplete penetrance of 35–40% and have simply been associated with dry skin as well as more severe manifestations of ichthyosis vulgaris. It is thought that functional similarities of other genes in the epidermal differentiation complex may mitigate the negative effect of filaggrin mutations.

Once the association of ichthyosis vulgaris to filaggrin was solidified, this led to the consideration that filaggrin might play a role in atopic eczema as well. There is significant overlap of these diseases, with 35–40% of individuals afflicted by ichthyosis vulgaris also suffering from atopic disease. In one study, 49% of ichthyosis patients with filaggrin mutations suffered from atopic eczema, while only 10% of ichthyosis patients suffered from asthma [8]. The drive to investigate a link between filaggrin and atopic disease, and atopic eczema in particular, was further encouraged by previous studies demonstrating linkage of atopic eczema to the region of the epidermal differentiation complex [9].

Filaggrin in atopic dermatitis

Atopic eczema (atopic dermatitis) is a chronic inflammatory skin disorder that affects approximately 15–20% of children world wide, with significant impairment of quality of life attributable to the associated symptoms and secondary infections. It is also considered the first step of the atopic march that is associated with later development of allergic rhinitis and asthma. Family history of eczema or atopy is highly predictive of increased risk of developing atopic eczema [10]. The two most common filaggrin null mutations (R501X and 2282del14) have been the most reproducible genetic factor in many large European cohorts for increased risk of eczema. Overall, between 15–20% of atopic eczema patients of European decent are estimated to carry a mutation in filaggrin. Further indicative of the clinical significance of the function of this protein, filaggrin null mutations have been identified in as many as 50% of patients with moderate to severe eczema in European populations and 20% in Asian populations [5,11]. The identified mutations are all nonsense mutations that either prevent or significantly decrease filaggrin expression in the skin. Development of eczema in filaggrin carriers is not dependent upon allergic sensitization. However, the odds ratio for atopic eczema as compared with nonatopic eczema is somewhat higher [12,13].

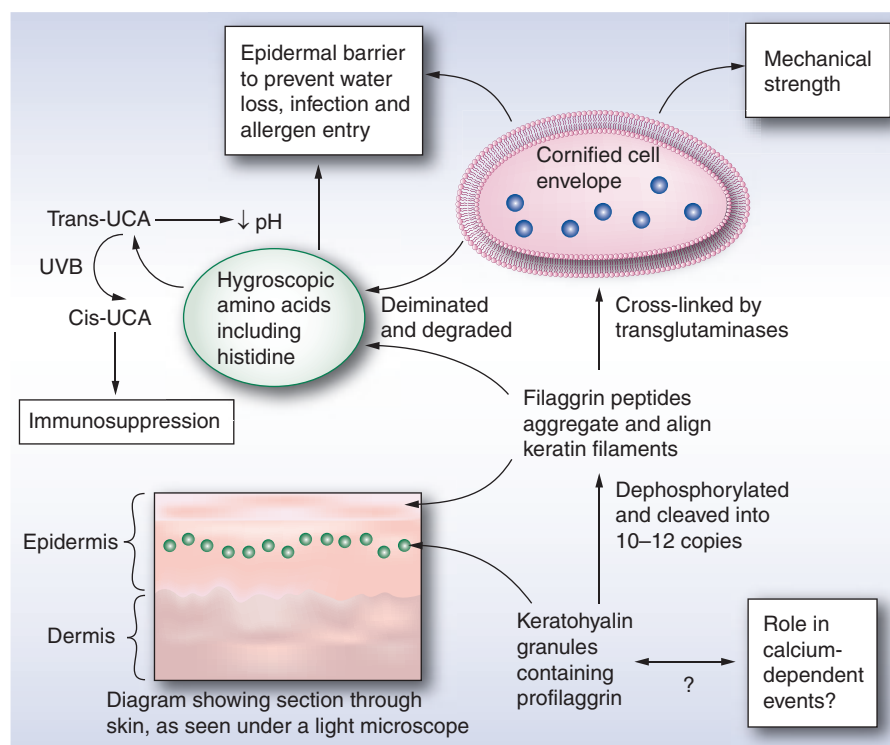


Figure 2. Role of filaggrin in maintaining skin barrier function.

UCA: Urocanic acid; UVB: Ultraviolet B.
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In the largest study to date of over 3000 European children of German descent, presence of one of five known filaggrin null alleles increased the risk of eczema, particularly atopic eczema (odds ratio [OR]: 4.5) [7]. In this study, associations were observed between filaggrin mutations and all three of the disease phenotypes (asthma, allergic rhinitis and atopic eczema) we associate with the atopic march. The children studied were 9–11 years of age. Skin prick testing was performed to six aeroallergens (*Dermatophagoides pteronyssinus*, *Dermatophagoides farina*, *Alternaria tenuis*, cat, mixed grass and tree pollen). Skin prick testing was determined to be positive if the wheal was 3 mm greater than the negative control. Atopic eczema was defined as a physician diagnosis of atopic dermatitis and positive skin prick testing to at least one aeroallergen. Asthma was defined as physician diagnosed asthma or at least two episodes of spastic bronchitis on a patient questionnaire and categorized as allergic if there was presence of at least one positive skin prick test to an aeroallergen. Allergic rhinitis was defined as physician diagnosed hayfever in the setting of at least one positive skin prick test. In addition, filaggrin null alleles were associated with increased risk of sensitization (positive skin test) and this was strongest in individuals with eczema [7].

In several studies describing patients with atopic eczema and filaggrin mutations, the eczema in patients with filaggrin mutation are typically more severe based on SCORAD assessment, presents early in life and often persists to adulthood. In addition, it manifests with hyperlinearity of the palms and is more strongly associated with asthma than in patients with eczema who do not

have these genetic abnormalities [13–15]. Skin natural moisturizing factor is of significantly different composition in patients with atopic eczema who carry filaggrin mutations versus those who do not carry the mutations [11].

There are many patients with severe atopic eczema who do not have any known mutations in filaggrin [1,7]. In these patients, there is evidence that the atopic response itself can modulate keratinocyte expression of filaggrin, particularly the expression of prototypical Th2 cytokines. *In vitro* incubation of keratinocyte with IL-4 and IL-13 in tissue culture led to decreased filaggrin expression. Conversely, IFN- γ coinubation led to increased filaggrin expression [16]. *In vitro* studies have also assessed filaggrin gene expression and histologic staining in samples taken from lesional skin versus healthy skin in atopic eczema patients with and without filaggrin mutations compared with healthy controls. In total 39 subjects were genotyped for the R501X and 228del4 mutations. Five were found to have the 228del4 mutation and of these three had atopic eczema. Filaggrin gene expression overall was lower in atopic eczema patients with or without the 228del4 mutation than in healthy controls who did not carry the mutation. In lesional skin from atopic eczema patients with the 228del4 mutation, the level of filaggrin expression was significantly lower than was seen in lesional skin for atopic eczema patients who did not carry the mutation. Histologic staining demonstrated decreased filaggrin staining in acutely involved skin in all atopic eczema patients, but the level was significantly lower in those with filaggrin mutations [16]. Taken together, these data indicate that although filaggrin mutations are significant, active atopic eczema alone can induce decreases in filaggrin expression, which would then have functional implications of impaired skin barrier integrity for all atopic eczema patients, not just those who carry filaggrin mutations. Filaggrin also effects susceptibility to bacterial and viral infection in individuals with atopic eczema. *In vitro*, growth of *Staphylococcus aureus* was inhibited when grown in the presence of urocanic acid and pyrrolidone carboxylic acid, the breakdown products of filaggrin, which are key components of natural moisturizing factor [17]. This is thought to be due to a relative decrease in culture pH caused by these acids. In addition, the ability of the staphylococcal organisms to secrete clumping factor B and fibronectin binding proteins was diminished by the addition of these acids to the culture media. Clumping factor B allows bacterial adhesion to cells of the cornified envelope by binding to cytokeratin 10, while fibronectin binding protein allows bacterial binding to the increased levels of fibronectin found in the skin of atopic eczema patients.

The concentration of urocanic acid and pyrrolidone carboxylic acid are known to be lower in patients with filaggrin null mutations [18]. Since filaggrin expression is also reduced in lesional skin in patients with atopic eczema without filaggrin mutations, the skin pH changes attributable to urocanic acid and pyrrolidone carboxylic acid are probably as applicable to these patients as well. Overall, filaggrin mutations contribute to the increased risk of staphylococcal skin infections in patients with atopic eczema as a whole by allowing more growth and better adherence of the organisms to the skin surface [17]. Once colonized, *Staphylococcus*

can then act as a superantigen to perpetuate the T-cell-mediated inflammation characteristic of moderate-to-severe atopic eczema.

Eczema herpeticum is a severe complication of herpes simplex infections seen in atopic eczema patients. There is an increased risk of eczema herpeticum in European and African patients with the two most common *FLG* mutations, R501X and 228del4 (OR: 10), although the 228del4 mutation was not observed in any of the African patients [19]. Eczema vaccinatum, the human syndrome of disseminated vaccine virus following smallpox vaccination, is a life-threatening complication in patients with atopic eczema. In filaggrin deficient mice, there was an increased risk of disseminated vaccinia virus following vaccination with an attenuated virus that was associated with IL-17-mediated inflammation [20]. Taken together these studies indicate that carriers of filaggrin mutations are at increased risk of severe viral complications of atopic eczema.

Patients with filaggrin null mutations have been shown to have increased transepidermal water loss (TEWL) even prior to the onset of clinical cutaneous disease, supporting the concept that the barrier dysfunction is induced by poor filaggrin function rather than inflammation causing the skin barrier defects [21]. In addition, the degree of TEWL correlated with the severity of the patients' eczema as determined by SCORAD [22,23]. This is not surprising, since dry skin is inherently more pruritic. As patients experience more pruritus, they will reflexively scratch more, perpetuating the itch-scratch cycle that drives poorly controlled atopic eczema.

Skin barrier function in allergic sensitization & the atopic march

Defects in skin barrier function are thought to allow transcutaneous sensitization to both food and environmental allergens. Barrier dysfunction is also associated with systemic responses such as increased total IgE and airway hyperreactivity, possibly due to these sensitizations. In mouse models of filaggrin null mutations increased transcutaneous sensitization has been demonstrated [24,25]. There is an increased risk (OR: 1.6) of allergic sensitization in filaggrin carriers with a history of eczema. Atopic eczema severity as determined by SCORAD has been found to have a correlation with the specific IgE for house dust mite allergen and cat dander in patients with filaggrin null mutations. This correlation has not been seen in atopic eczema patients with wild-type filaggrin alleles [22]. The R501X mutation is associated with higher serum IgE levels overall when compared with the other common mutation 228del4 due to a higher rate of penetrance [10].

Allergic sensitization was associated with filaggrin mutations only in those patients who had also had eczema [7,12]. It therefore follows that in infants with severe eczema with defects in epidermal barrier function, such as that induced by filaggrin mutation, there is an increased risk of allergic sensitization. Maintaining and repairing defects in the epidermal barrier are likely to be crucial in atopy prone individuals to prevent progression through the atopic march.

The common immunologic features of atopic disease, including elevations in IgE and IgE allergen sensitization, Th2 cytokine skewing, eosinophilia and epithelial dysfunction led to the theory that these diseases represent a progression disease that has its root in a shared initiating insult. The atopic march is the purported

stepwise progression of patients' atopic disease development over the course of a lifetime. According to the atopic march theory, atopic disease first presents with the presentation of atopic eczema in infancy. If left unchecked, the disease process then permits further atopic development due to atopic sensitization with clinical presentations of allergic rhinitis and allergic asthma. This is supported by observations that the incidence of eczema peaks in the first years of life then declines while incidence of asthma and allergic rhinitis are less common in infancy but their incidence increases with age. In addition, the risk for developing asthma correlates with the severity of atopic eczema. While 30% of patients with mild eczema go on to develop asthma, in patients with severe eczema 70% go on to develop asthma [26].

In one cohort of over 1000 children followed from birth until 7 years of age, 21% experienced atopic eczema in the first 2 years of life. In these children with early onset of atopic eczema and allergic sensitization to wheat, cat, dust mite, soy or birch there was an association with early wheezing, which was categorized as any episodes of wheezing under age 3 years. Children with this combination of symptoms experienced more significant overall impairment in lung function [27]. In another cohort of 94 children with atopic eczema followed prospectively for the first 7 years of life, 43% went on to develop asthma and 45% went on to develop allergic rhinitis. Severe eczema in infancy was associated with increased rates of food specific IgE and early onset eczema was associated with an increased risk of inhalant sensitization [28]. There are also animal models supportive of the association between skin barrier dysfunction, atopic eczema and later development of other atopic disease.

Tape stripping followed by allergen application to the skin of mice induced IgE, IgG1 and IL-4 expression as well as a predominantly eosinophilic infiltrate. Conversely, application of allergen to intact skin led to Th1 skewing in draining lymph nodes. This supports the concept that disruption in the barrier function of the skin can predispose to a development of a typical atopic milieu of IgE and Th2-type cytokine production [29]. In tape-stripped mice exposed to ovalbumin epicutaneously, ovalbumin specific IgE was detectable, indicating that sensitization had occurred. This was not seen in mice exposed to ovalbumin via intraperitoneal injection. When the epicutaneously exposed mice were later challenged with inhaled ovalbumin, there was a significant increase in eosinophils in bronchoalveolar lavage obtained after the challenge [30].

In the skin, the Notch pathway is a key signaling pathway in the organization of skin keratinocyte structural organization. In mice with a keratinocyte specific knock-down of the Notch signaling pathway, there was a phenotypic expression of chronic skin barrier defect with increased transepidermal water loss and development of a skin disorder similar to eczema [31]. The genetically engineered mice had increased IgE levels and systemic Th2 cell expansion. These mice were also found to have increased expression of thymic stromal lymphopoietin (TSLP) in their keratinocytes and had increased susceptibility to allergic asthma characterized by severe bronchospasm response to ovalbumin challenge, which was not seen in wild-type controls. However, when the TSLP receptor was deleted, the development of asthma as defined by ovalbumin-induced bronchospasm

was blocked. Further, in outbred transgenic mice that continued to have high TSLP expression but relative absence of a skin barrier defect, a severe asthma phenotype developed [31]. Taken together these data suggest a critical role for TSLP in the progression of the atopic march from atopic eczema to allergic asthma.

TSLP is an IL-7-like cytokine, produced in epithelial cells of the skin, lung and gut that has been associated with atopic eczema and allergic asthma. When antigen-presenting cells, particularly dendritic cells, are cocultured with T cells in a TSLP-rich media, the ensuing T-cell response is predominantly of the Th2 polarization, with an increased expression of IL-4, IL-5, IL-13 and TNF- α . TSLP can also independently directly promote naive T-cell differentiation toward to Th2 phenotype in culture [32]. Human epithelial cells from lesional skin of atopic eczema patients have been found to express higher levels of TSLP than healthy skin and non-lesional skin of atopic eczema patients [33]. However, it is important to note that it has not yet been demonstrated that TSLP serum concentrations are elevated in atopic individuals compared with healthy controls. It is theorized that the increased TSLP expression of keratinocytes leads to an overall Th2 skewing in patients with atopic eczema, which then feeds into the cycle of the atopic march to promote the development of allergic rhinitis and allergic asthma.

Filaggrin in airway disease: allergic rhinitis & asthma

Since approximately 40% of infants with atopic eczema later develop asthma and/or allergic rhinitis, the next logical quest was the search to determine if there is an association between filaggrin and airway atopic disease. Filaggrin is not expressed in the mucosal surfaces of the nose, upper airway or lungs, therefore it would seem unlikely to demonstrate an association between decreased expression of this protein and development of allergic rhinitis and allergic asthma. Somewhat surprisingly then, one of the initial studies to assess association of the R501X and 2282del4 mutations with susceptibility to atopic disease found significant association between the presence of these mutations with the phenotype of allergic rhinitis and eczema and allergic sensitization plus eczema [12]. However, in the same large study of German children discussed previously, filaggrin mutations were associated with a twofold increased risk of allergic rhinitis irrespective of prior atopic eczema history [7]. This association was dependent upon atopic eczema history in another large cohort [14].

Filaggrin has also been proffered as a major genetic risk factor for development of allergic asthma. Patients with either of the filaggrin null alleles have been demonstrated to have increased need for asthma medication and greater obstructive lung function impairment [34]. In addition, mild persistent asthma patients who are carriers of a filaggrin null allele have a nearly twofold higher risk of exacerbation with need for oral steroid therapy compared with asthma patients with wild-type filaggrin alleles [35]. This association is strongest when the patients have both asthma and atopic eczema. One flaw in the studies assessing risk of asthma has been the fact that subjects were typically selected on the basis of a prior diagnosis of atopic eczema. It is well established that infants with atopic eczema are at a much higher risk of later developing asthma in the progression of the atopic march described earlier. When

asthma was used as the inclusion criteria irrespective of atopic eczema history, the strength of association with filaggrin mutations drops notably [13]. However, this may reflect the heterogeneity of patients diagnosed with asthma.

These associations are thought to be primarily due to transcutaneous sensitizations to allergens through the impaired skin barrier of skin affected by atopic eczema. The presence of the allergic sensitization leads to an overall Th2 type skewing, which then triggers asthma and rhinitis symptoms triggered by these allergens. But this cannot be the only link between allergic rhinitis, asthma, eczema and filaggrin mutations as some patients developed symptoms in the absence of demonstrable allergic sensitization [7].

Filaggrin in food allergy & gastrointestinal disease

In studies of the eosinophilic esophagitis transcriptome, the highest concentration of genetic abnormalities have been described in the area of the epidermal differentiation complex, where filaggrin is located. In addition, patients with eosinophilic esophagitis commonly also suffer from atopic dermatitis. Thus far no significant differences in filaggrin expression have been noted in patients with atopic eosinophilic esophagitis as compared with those eosinophilic esophagitis patients without atopic sensitization. However, filaggrin mutations are associated with increased risk of eosinophilic esophagitis overall [36].

Patients with filaggrin null mutations have been found to have an increased rate of peanut sensitization, with an OR of 3.8 when controlled for the presence of atopic dermatitis [37]. Transcutaneous sensitization early in life in the absence of enteral exposure has been postulated to be one potential etiology of the increased rate of peanut sensitization and resultant allergy [38].

Filaggrin in immunodeficiency

Profilaggrin is degraded by several serine proteases to produce active filaggrin protein. Serine protease inhibitor *SPINK5* loss of function mutations are associated with Comel Netherton syndrome. Loss of inhibition of the serine proteases would lead to unchecked degradation of profilaggrin and relative filaggrin deficiency due to constant consumption. These mutations also lead to increased TSLP expression in the skin and airway epithelium. Patients with Comel Netherton syndrome suffer from a severe form of atopic eczema with elevated IgE and atopic disease as well as recurrent skin infections, predominantly due to *S. aureus*. Interestingly, a small cohort of these patients have recently been found to have poor specific antibody function and demonstrated a good clinical response to treatment with intravenous immunoglobulin [39].

Therapeutic changes based on knowledge of filaggrin function

Filaggrin is a crucial component of the barrier function in the layers of the skin and epithelium where it is found. Our recent advances detail the absence or decreased presence of this protein in patients with atopic disease both with and without mutations in the filaggrin gene. These genetic or functional deficits lead to the rather obvious conclusion that therapy aimed to address this defect must address barrier dysfunction first and foremost.

Atopic eczema is traditionally perceived as an immunologic disease, to be treated with immunosuppression. However, the association of decreased barrier function with increased risk of allergic sensitization and bacterial and viral infection associated with filaggrin mutations supports the growing body of evidence that effective therapy must enhance skin barrier function. In the skin this will mean aggressive proactive, rather than reactive, use of topical compounds to enhance barrier function, which will restore normal skin pH and minimize risk of allergic sensitization and infection [40]. Infectious agents such as herpes virus and *S. aureus* are common triggers for flares and must also be actively controlled. As we reviewed previously, filaggrin mutations are associated with decreased resistance to these organisms.

Moisturization is an important component of barrier function as evidenced by the increased TEWL seen in patients with filaggrin mutations. Basic cleansing is important and use of a pH balanced (pH = 6) cleansing product can be helpful to restore skin to its normal physiologic acid–base balance, which is crucial in the host defense role of the skin barrier. In an effort to control staphylococcal infection and superantigen stimulation causing severely flared atopic dermatitis, dilute bleach baths are also often recommended, as is use of topical antimicrobial agents such as mupirocin [41]. After the bath unscented and dye free emollients should be applied immediately, while the skin is still damp. Avoidance of products containing perfumes and alcohols is also crucial as these components can contribute to further drying and irritation of the skin. Lipid containing emollients help to supplement the barrier dysfunction of the skin [42]. Emollients are recommended to be used at least twice a day with increasing frequency as needed to keep the skin moist and limit pruritus. A typical child will require 150–200 g of emollient on a weekly basis and adults may require up to 500 g. Emollients containing potential allergens such as peanut, oat, shea nut should be avoided since the skin can serve as entry for allergic sensitization [43]. A recent pilot study of 15 atopic dermatitis patients using a hydrogel compound under occlusion demonstrated similar efficacy to use of triamcinolone 0.1%, supporting the concept that restoration of barrier function is an imperative treatment objective [44].

Since the inherent problem of the filaggrin mutations is chronic skin barrier dysfunction that is not resolved with resolution of an acute flare, a proactive approach to minimize future exacerbations has been proposed [40]. Acute flares of atopic dermatitis are typically treated in the traditional fashion, with higher potency topical steroids and removal of identifiable triggers. Once acute flares are resolved, prophylactic twice-weekly application of either topical steroids or calcineurin inhibitors in areas of previous flare can be beneficial to achieve optimal of chronic atopic eczema. Prophylactic use of fluticasone 0.05% four-times weekly for 4 weeks followed by twice-weekly for 16 weeks was associated with a significant decrease in atopic eczema relapse compared with placebo without evidence of skin atrophy [45]. Tacrolimus 0.1% ointment has also been used for twice-weekly prophylaxis with significant decrease in frequency of exacerbations and significant improvement in quality of life. These regimens have been used in patients with varying severity of disease with similar benefits [40]. These prophylactic regimens may decrease the overall burden of

exposure to high potency steroids by allowing for a stable steady state of control of atopic eczema as a chronic disease.

Treatment of atopic eczema with pimecrolimus compared with β methasone led to similar improvements in TEWL, filaggrin expression and decreased dye penetration, which was used as a proxy for functionality of the outside–inside barrier and risk of transcutaneous sensitization [46]. However, β methasone was associated with a more significant decrease in epidermal proliferation, which was also associated with epidermal thinning. The effects of β methasone were primarily attributed to its reduction in inflammation and blood flow. While this is beneficial in the short term, the long-term risk of skin atrophy is substantial. Pimecrolimus was associated with regular lipid bilayers, which are a key component to normal barrier function of the skin. This, when added to the other shared benefits with high potency topical steroids, may make pimecrolimus and the calcineurin inhibitors as a whole better suited to the long-term therapy needed to address barrier dysfunction in most patients with atopic dermatitis [46]. Topical pimecrolimus has been shown to be both safe and effective in infants as young as 3 months of age [46]. However, the use of pimecrolimus 1% cream in this study did not lead to a decreased rate of later atopic disease development such as allergic rhinitis or allergic asthma. The more rapid reduction in symptoms and measurable parameters associated with topical steroids, however, supports their continued use for acute flares. Effective treatment of inflammation can also help to decrease staphylococcal carriage [47].

As filaggrin expression has been demonstrated to be affected *in vitro* by Th2-type cytokines it is reasonable to also consider the possibility that therapies aimed at these cytokines and T cells overall might also be important emerging realms for therapeutic intervention. Unfortunately clinical trials utilizing mepolizumab (anti-IL-5) have not demonstrated a clinical benefit for atopic eczema. However, Alefacept, which targets CD2 on T cells has had mixed but somewhat promising results. Clear clinical benefit and reduction in cytokine production was seen in one group of ten atopic dermatitis patients treated over a period of 12 weeks. There was a trend towards clinical improvement in a group of nine atopic dermatitis patients treated for 16 weeks. Efalizumab, which targets CD11a and overall leukocyte migration, has also shown some promise in early clinical trials [42]. In theory these targeted agents would seem likely to confer great benefit with reduced risk compared with more global effects of other immunosuppressives such as corticosteroids. However the fact that they require systemic administration and therefore have systemic effects can pose a significant hazard. The benefits of the latter two agents must be tempered by their risk since they both have systemic immunosuppressive effects. In addition, the mechanisms by which monoclonal biologic products are expected to have their benefit are clearly not the only means by which allergic inflammation occurs and this redundant ability of the immune system may also limit the practical use of these treatment modalities.

Expert commentary & five-year view

Over the next 5 years we would expect to see mutation analysis as a whole playing a larger role in the diagnostic evaluation and risk

stratification for patients suffering from a broad range of allergic disease. Identification of those patients with defects in filaggrin protein expression will allow us to focus even more heavily on barrier enhancement methodologies. It is possible that therapeutics aimed at specifically enhancing the skin's barrier function by improved moisture retention, decreased transcutaneous sensitization and limitation of bacterial overgrowth will halt the atopic march at the stage of atopic eczema. In addition, we would expect to see increased development and use of biologics aimed at disruption of Th2 skewing of naive T-cell differentiation. This may then be associated with lower rates of other atopic diseases, including allergic rhinitis, asthma and food allergy in patients who carry filaggrin null mutations.

Over the last 5 years, filaggrin loss of function mutations have emerged as a significant genetic marker for eczema that can be expected to be more severe, more highly associated with allergic sensitization and persist later in life than in patients with other forms of eczema. It is noteworthy that filaggrin expression is also lower in the lesional skin of atopic eczema patients without loss of function mutations. This is one indicator of a uniform disease pathway of impaired skin barrier function in all eczema patients, which underscores the growing understanding of the important role of the intact skin epithelium in prevention of allergic sensitization early in life. Treatments aimed at enhancing the skin barrier seem likely to mitigate the degree of allergic sensitization and decrease the morbidity associated with severe atopic dermatitis.

The theory of impaired barrier function and elevated TSLP expression is a hallmark of the atopic march hypothesis. Atopic eczema has long been implicated as the first step of the atopic march and further studies have linked filaggrin to increased risk for asthma in patients who suffer from concomitant atopic eczema, as well as allergic rhinitis and peanut allergy. These other atopic diseases are thought to be due to transcutaneous sensitization to both food and aeroallergens, which then allows development of lifelong atopic disease precipitated by predominant skewing of the naive T cells toward a Th2 phenotype. As we are better able to define the subgroups of asthmatic and allergic rhinitis patients into those who have truly progressed along the route of the atopic march, the associations between filaggrin loss of function mutations and other atopic disease may become more clear. It may be possible to use *FLG* sequencing and expression as biomarker for disease control. By addressing the barrier dysfunction of atopic dermatitis proactively with the use of effective and preventative emollients and targeted use of immunomodulators, it is possible that we may optimize our patients' chances to stop the atopic march, lower their overall burden of atopy and enhance their quality of life.

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Key issues

- Filaggrin mutations are associated with barrier defects in the skin epithelium and the development of atopic dermatitis.
- Dysfunction of the skin epithelium has been proposed as the initial permissive step in allergic sensitization. Once sensitization occurs, it appears that the development of atopic diseases becomes more likely, since patients with filaggrin mutations and atopic dermatitis are also at increased risk of developing asthma and allergic rhinitis.
- Filaggrin mutations do not seem to be as clearly correlated with allergic rhinitis and asthma in the absence of atopic dermatitis.
- Regular, frequent use of emollients to improve moisturization and aggressive treatment with topical corticosteroids to control atopic dermatitis appears to be more effective than topical steroids alone.

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