

Causes of epidermal filaggrin reduction and their role in the pathogenesis of atopic dermatitis

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The epidermis protects human subjects from exogenous stressors and helps to maintain internal fluid and electrolyte homeostasis. Filaggrin is a crucial epidermal protein that is important for the formation of the corneocyte, as well as the generation of its intracellular metabolites, which contribute to stratum corneum hydration and pH. The levels of filaggrin and its degradation products are influenced not only by the filaggrin genotype but also by inflammation and exogenous stressors. Pertinently, filaggrin deficiency is observed in patients with atopic dermatitis regardless of filaggrin mutation status, suggesting that the absence of filaggrin is a key factor in the pathogenesis of this skin condition. In this article we review the various causes of low filaggrin levels, centralizing the functional and morphologic role of a deficiency in filaggrin, its metabolites, or both in the etiopathogenesis of atopic dermatitis. (*J Allergy Clin Immunol* 2014;■■■:■■■-■■■.)

Key words: Atopic dermatitis, barrier function, filaggrin, histidine, ichthyosis vulgaris, lamellar body, pathogenesis, stratum corneum, T_H2 cytokines, urocanic acid

The epidermis protects human subjects from exogenous stressors and helps to maintain internal fluid and electrolyte homeostasis. A crucial epidermal protein is filaggrin, which is important for the formation of the corneocyte, as well as the generation of its intracellular metabolites, which contribute to stratum corneum (SC) hydration and pH while also interdicting photons of UV-B irradiation.¹ Loss-of-function mutations in the filaggrin gene (*FLG*) result in either a reduction (heterozygous) or complete absence (homozygous) of epidermal filaggrin and its degradation products.^{2,3} *FLG* mutations cause ichthyosis vulgaris (IV)^{2,4} and constitute the strongest known risk factor for atopic dermatitis (AD) in Northern European and Asian subjects, in whom they are associated with an early onset, a more severe course, and a higher prevalence of IgE-mediated

Abbreviations used

AD: Atopic dermatitis
CE: Cornified envelope
FLG: Filaggrin gene
ILC: Innate lymphoid cell
IV: Ichthyosis vulgaris
NMF: Natural moisturizing factor
SC: Stratum corneum
SP: Serine protease
TJ: Tight junction
TSLP: Thymic stromal lymphopoietin
ZO: Zonula occludens

sensitization, so-called extrinsic AD.⁵⁻⁹ Recent review articles have discussed the role of *FLG* mutations in dermatologic and allergic disease.^{1,4,6,10} Notably, epidermal filaggrin deficiency is observed in patients with AD, regardless of *FLG* mutation status, with the conclusion being that absence of filaggrin is key in the pathogenesis of AD.¹¹

Epidermal filaggrin expression is downregulated by T_H2 cytokines in patients with AD,¹² and filaggrin proteolysis is accelerated after exposure to either low ambient humidity^{13,14} or skin irritants.^{15,16} Thus levels of filaggrin and its degradation products are influenced not only by the filaggrin genotype but also by inflammation and exogenous stressors. These observations support both an “outside-to-inside” AD pathogenetic hypothesis, which postulates that AD develops because of a primary skin barrier abnormality, with immunologic alterations representing downstream phenomena, and an “outside-to-inside-and-back-to-outside” AD pathogenesis, in which secondary immunologic activation will result in further stress on the barrier.¹⁷

Here we review the various causes of low filaggrin levels, centralizing the functional and morphologic role of a deficiency in filaggrin, its metabolites, or both in the etiopathogenesis of AD. Although these mechanisms place filaggrin at the epicenter of AD etiopathogenesis, we acknowledge that mutations in *FLG* constitute only 1 important inherited contributor to AD risk and that many other factors contribute to AD pathogenesis.^{11,18-21}

FILAGGRIN IN NORMAL EPIDERMIS

Although still present in keratohyalin granules of the stratum granulosum, 400-kDa profilaggrin polymers are released, proteolytically cleaved, and then dephosphorylated into 10 to 12 identical filaggrin monomers by enzymes, such as furin, endoprotease 1, calpain 1, matriptase, and elastase 2.^{1,22-25} The liberated filaggrin monomers aggregate keratin filaments into tight bundles, resulting in collapse and flattening of corneocytes.²⁶ Additional cytosolic proteins, such as keratins

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1 and 10, also associate with the cornified envelope (CE) during terminal differentiation, constituting, along with filaggrin and its degradation products, the bulk of the cytosol of the anucleate corneocytes of the SC.²⁷ Filaggrin monomers, along with several other epidermal differentiation-linked proteins, are then cross-linked into the CE by transglutaminases.

In addition to the CE, an external monolayer of covalently bound ω -OH ceramides provides a scaffold on which extracellular lipids organize its lamellar bilayers. Glucosylceramides and phospholipids, previously deposited in the extracellular domains by means of lamellar body secretion, then transfer into arrays of lamellar bilayers after lipid-processing hydrolases, such as β -glucocerebrosidase, acidic sphingomyelinase, secretory phospholipase A₂, and steroid sulfatase, have generated ceramides, free fatty acids, and cholesterol, respectively.²⁸ Lamellar bodies also secrete antimicrobial peptides, proteases/antiproteases, and apolipoproteins into the extracellular matrix of the SC. This sequence results in a formidable protective shield of protein-enriched corneocytes (the “bricks”) surrounded by organized arrays of hydrophobic lipids (the “mortar”) that simultaneously prevents the outward movement of water and the inward movement of allergens and microbial pathogens.

In the outer layers of the stratum granulosum, the keratinocytes are linked by tight junction (TJ) proteins that regulate the transport of macromolecules through the extracellular space.²⁹ Three main transmembrane proteins exist in TJs: occludin, claudin, and junctional adhesion molecules. The zonula occludens (ZO) 1 and ZO-2 proteins and cingulin provide direct links to the actin cytoskeleton.³⁰

As corneocytes move apically through the SC, filaggrin detaches from the CE, followed by its proteolytic degradation within the cytosol into its constituent amino acids, including glutamine, arginine, and histidine, as well as their downstream deiminated metabolites.¹ Cleavage of the filaggrin monomers is accomplished by caspase-14, calpain 1, and bleomycin hydrolase.¹ Histidine, which comprises approximately 10% of the amino acids in filaggrin, is a substrate for histidase (histidine ammonia-lyase), which generates *trans*-urocanic acid, a major UVB-absorbing epidermal chromophore that also contributes to the acid mantle of the skin.¹ Another abundant amino acid, glutamine, is further converted into pyrrolidone-5-carboxylic acid, a major component of the natural moisturizing factors (NMFs) of the SC and therefore a potent humectant, accounting for much water retention in the SC.

As we will develop further below, filaggrin degradation products affect multiple functions that are crucial for the maintenance of epidermal homeostasis, including not only UV defense, SC hydration, and acidification, which in turn inhibits colonization by pathogenic microorganisms, but also release of IL-1 α and IL-1 β .³¹ IL-1 α and IL-1 β are essential proinflammatory cytokines that stimulate the initial acute phase of inflammation.³² Notably, IL-1 α induces the expression of adhesion molecules on endothelial cells, facilitating the infiltration of inflammatory and immunocompetent cells into the stressed tissue.³³

GENETIC CAUSES OF REDUCED FILAGGRIN LEVELS

Loss-of-function mutations in 1 or both alleles of *FLG* result in reduced or completely absent levels of epidermal filaggrin, respectively (Table I)^{2,3,11-16,34-52} Approximately 10% of

Northern European subjects from the general population are heterozygous mutation carriers, and approximately 0.1% are homozygous.⁴ The prevalence of *FLG* mutations in Chinese, Japanese, and Korean subjects reaches 3% to 6%.⁴ Although there are considerably fewer studies from Southern European and African populations, published studies to date suggest that *FLG* mutations are uncommon or even absent.⁵³⁻⁵⁶ However, in certain Northern European populations with AD, the prevalence of *FLG* mutations is much higher, affecting between 25% and 50%.⁸ It has been proposed that common *FLG* mutations might have evolved to generate vitamin D in light-pigmented northern subjects^{57,58} or, alternatively, to increase penetration of microorganisms as an *in vivo* “vaccinating” mechanism,¹⁰ factors that both could have evolved to enhance the survival of modern human subjects.

Intragenic copy number variations exist in *FLG*, with 33.9% of normal Irish persons having 10 repeats, 51.5% having 11 repeats, and 14.6% having 12 repeats.³⁴ The number of *FLG* repeats correlates significantly with SC UCA levels and the risk of xerosis and AD.^{34,35} Epigenetic regulatory mechanisms are mitotically heritable and can alter gene expression without changing the DNA sequence. Accordingly, one recent study showed that DNA methylation in one adjacent CpG site of *FLG* significantly increased the risk of AD.³⁶

Whether genetic variations in enzymes involved in profilaggrin synthesis and polymer-to-monomer processing can also affect the levels and functions of filaggrin and its metabolites remains unknown but seems likely.

ENVIRONMENTAL CAUSES OF REDUCED FILAGGRIN LEVELS

There is sparse information available about environmental conditions and exogenous stressors that can reduce epidermal filaggrin levels (Table I). However, monomeric filaggrin is hydrolyzed in a humidity-sensitive fashion as environmental humidity decreases and after a rapid shift from a humid to a dry environment.¹⁴ The higher prevalence of AD in American children residing in areas with low relative humidity, low mean temperature, and more days of central heating could be explained by the negative effects of acquired filaggrin deficiency on skin barrier function.⁵⁹ Pertinently, Danish children with AD and *FLG* mutations have dermatitis localized to air-exposed skin areas, such as the cheeks and dorsal aspects of the hands, significantly more often when compared with wild-type children with AD,⁶⁰ suggesting the effect of climatic exposure on AD susceptibility.

Although there is little information about the effects of solar radiation on filaggrin, potent single doses of UVB irradiation *in vitro* lead to downregulation of filaggrin expression.³⁷ Even physiologic doses of UVB irradiation of normal human skin grafted onto nude mice reduce filaggrin immunostaining in the stratum granulosum.⁶¹

Not only climatic influences but also exposure to irritants and perhaps even water can also reduce epidermal filaggrin levels.^{15,16} Profilaggrin expression decreased after experimental applications of 1% sodium lauryl sulfate under occlusion for 24 hours.¹⁵ Whether mechanical damage, such as that caused by scratching, can affect human filaggrin expression/degradation has not been studied but seems likely based on the following scenario. In normal epidermis a calcium gradient exists, with low calcium levels in the basal and spinous epidermal layers and increasing calcium levels toward the outer stratum

TABLE I. Factors that can reduce levels of epidermal profilaggrin, filaggrin, or both

- *FLG* alterations
 - Mutations^{2,3}
 - DNA methylation³⁶
 - Copy number variations^{34,35}
- Environmental exposures
 - Low humidity^{13,14}
 - Sunburn³⁷
 - Skin irritants^{15,16}
 - Tape stripping⁴⁵
 - Psychological stress⁴⁶
 - Pruritus → excoriations → mechanical damage
 - Age⁴⁷
 - Water¹⁵
- Cytokines
 - IL-4, IL-13, IL-17A, IL-22, IL-25, IL-31, TNF- α ^{11,12,39-44}
- Microorganisms
 - Human papilloma virus⁴⁸
 - Tinea corporis (*Trichophyton rubrum*)⁴⁹
 - Propionibacterium acnes* (JCM 6473)⁵⁰
- Topical therapy
 - Dithranol⁵¹
 - Retinoic acid⁵²
 - Corticosteroids (long term)³⁸

This list is not intended to be all inclusive.

granulosum, which again decrease across the SC. Acute barrier perturbations, such as from scratching, can disrupt the calcium gradient, resulting in reduced calcium levels in the stratum granulosum and in turn downregulating filaggrin expression. Finally, topical corticosteroids, but neither tacrolimus nor coal tar, if used for a prolonged time, reduce epidermal filaggrin content.^{38,62}

INFLAMMATION-DRIVEN REDUCTIONS IN FILAGGRIN LEVELS

T_H2 skin inflammation, as observed in patients with AD, downregulates filaggrin, NMF, and caspase-14 levels in lesional and nonlesional skin, apparently independent of *FLG* mutation status.^{11,12,39-44} In contrast, the T_H1-related cytokine IFN- γ appears to significantly augment filaggrin expression, potentially balancing T_H2 skewing in patients with AD.¹² Although the role of the innate immune system on filaggrin expression has not yet been thoroughly investigated, activation of innate immune receptors results in the release of various inflammatory mediators from keratinocytes, which could then secondarily decrease filaggrin expression.⁶³ Interestingly, Toll-like receptor 2 agonists enhance the expression of TJ proteins,⁶⁴ which could potentially influence filaggrin levels indirectly, because TJ and filaggrin seem to interact, as described below. Collectively, these observations provide evidence for the operation of multiple “inside-outside” pathomechanisms in AD, where T_H2 skin inflammation can compromise skin barrier function. Finally, serum IgE can function as an autoantibody against some epidermal proteins in patients with AD,⁶⁵ but it is unknown whether IgE targets filaggrin as well.

FILAGGRIN DEFICIENCY AFFECTS EPIDERMAL PROTEIN EXPRESSION AND ORGANIZATION

Inherited and acquired filaggrin deficiency modifies both the intracellular and extracellular architecture of keratinocytes, as

well as the normal physiology of the epidermis. At the light-microscopy level, the SC is thicker than normal, and the granular cell layer is either completely absent or strongly reduced in patients with IV or AD.⁴ At the ultrastructural level, the cytoskeleton of granular cells shows perinuclear retraction, and the distribution of both corneodesmosomes and TJs appears altered.^{66,67} Profilaggrin-deficient “flaky tail” mice display a reduction of epidermal growth factor receptors, as well as reduced E-cadherin, occludin, and loricrin expression.⁶⁸ Although loricrin expression appears not to be affected by *FLG* mutations in human subjects, expression of the TJ proteins occludin and ZO-1 is reduced in the epidermis of heterozygous and particularly homozygous *FLG* mutation carriers.⁶⁷

FILAGGRIN DEFICIENCY INTERFERES WITH LIPID SECRETION

Because of the cytoskeletal abnormalities described above, cargo loading into lamellar bodies is also impaired, and secretion is partially compromised in patients with IV and AD, resulting in entombment of some lamellar bodies within corneocytes.⁶⁷ Although impaired secretion appears to reduce the normal quantities and organization of the extracellular lamellar bilayers,⁶⁹ there also appear to be further abnormalities in free fatty acid and ceramide chain length in patients with AD independent of *FLG* mutation status.^{67,70} Thus secreted IFN- γ in patients with chronic AD downregulates the 2 fatty acid elongases ELOVL1 and ELOVL4, which are required to generate the very long-chain N-acyl fatty acids in ceramides and free fatty acids.⁷¹ The increased proportion of shorter-chain fatty acids results in lipid disorganization that likely further compromises barrier function in patients with AD.^{70,71} Yet a compensatory increase of lipid synthesis has been observed in patients with IV,⁷² and filaggrin deficiency *in vitro* results in a 2-fold increase in free fatty acid content caused by upregulation of secretory phospholipase A₂ IIA.⁷³ Interestingly, it was recently shown that mutations in (1) the fatty acid transfer protein 4 (*FATP4*) gene, which codes for the fatty acid transporter FATP4 in the suprabasal layer of the epidermis,⁷⁴ and (2) the *Tmem79* gene, encoding for a component in the lamellar granule secretory system,⁷⁵ both associate with an increased risk of AD. Although not associated with filaggrin deficiency *per se*, these observations emphasize the importance of altered lipid production and secretion in the pathogenesis of AD.

FILAGGRIN DEFICIENCY INCREASES SKIN PH, ACTIVATING SERINE PROTEASES THAT COMPROMISE BARRIER FUNCTION

The skin of *FLG* mutation carriers shows reduced hydration and increased transepidermal water loss caused by reduced levels of filaggrin metabolites, including NMFs, which regulate not only SC hydration but also the pH of the SC.^{76,77} The reduction in NMF increases the normally acidic SC pH with an allele dose-dependent effect whereby homozygous *FLG* mutation carriers have the highest pH levels.⁶⁷ Yet a compensatory upregulation of the sodium-hydrogen antiporter seems to counteract this pH increase,⁷³ perhaps explaining why an increase in skin surface pH is not always apparent in *FLG* mutation carriers.⁷⁸ An acidic SC milieu facilitates synthesis of ceramides by acidic sphingomyelinase and β -glucocerebrosidase while providing

resistance against growth of pathogenic microorganisms (see below).⁷⁹ Moreover, a sustained increase in pH leads to degradation of the ceramide-generating enzymes acidic sphingomyelinase and β -glucocerebrosidase.⁸⁰

Importantly, pH-sensitive serine proteases (SPs), or kallikreins, are activated after the pH increase observed in patients with AD, with multiple negative consequences. They not only lead to compromised intercellular connections caused by premature degradation of the corneodesmosomes, but SPs also activate IL-1 α and IL-1 β .^{80,81} The most compelling case for a key role of excess SP activity in the pathogenesis of AD comes from the study of Netherton syndrome, an autosomal recessive disorder caused by loss-of-function mutations in the *SPINK5* gene encoding the SP inhibitor, lymphoepithelial Kazal-type trypsin inhibitor type 1, which is strongly associated with AD. Here the magnitude of SP activation correlates with both the barrier defect and clinical severity and inversely with residual lymphoepithelial Kazal-type trypsin inhibitor expression.⁸⁰ Notably, the expression and activity levels of the endogenous proteases kallikreins 5, 7, and 14 in the skin are higher in flaky tail than in control mice,⁸² further emphasizing the critical role of excessive protease activity for filaggrin deficiency in AD pathogenesis.

PROTEASE ACTIVATION STIMULATES PRURITUS AND T_H2 INFLAMMATION IN PATIENTS WITH AD

In addition to the direct destructive effects of kallikreins, they also bind to the protease-activated receptor type 2,⁸⁰ which not only downregulates lamellar body secretion but also initiates a cascade of innate inflammatory responses, including activation of thymic stromal lymphopoietin (TSLP) protein from keratinocytes,⁸³ the master switch that initiates T_H2 inflammatory responses.^{83,84} TSLP is highly expressed in keratinocytes from lesional skin of patients with AD, and its secretion can be (indirectly) induced by exposure to allergens (eg, house dust mite), microorganisms, or mechanical injury.⁸⁴⁻⁸⁸ TSLP bears a nuclear factor κ B responsive element that might also respond to IL-1 β /IL-1 receptor stimulation, although a recent study suggests that IL-1 β does not activate TSLP.⁸⁹ Interestingly, TSLP causes cutaneous nerves to release neuropeptides that induce itch, which again leads to scratching, a further barrier deficit, and an increased risk of secondary bacterial colonization.⁹⁰

Moreover, innate lymphoid cells (ILCs), a recently identified family of heterogeneous immune cells, play a role in the pathogenesis of AD. Thus group 2 ILCs are enriched in lesional skin from patients with AD and appear to be critically dependent on TSLP stimulation but also respond to IL-25 and IL-33, resulting in secretion of T_H2 cytokines IL-5 and IL-13.^{91,92} Transgenic mice with a skin-specific overexpression of IL-33 have spontaneous AD-like disease with the activation of group 2 ILCs when bred under specific pathogen-free conditions.⁹³ Notably, the downregulation of E-cadherin in the setting of filaggrin deficiency indirectly stimulates group 2 ILCs to secrete T_H2 cytokines because of the absence of suppressive E-cadherin ligation.⁹² Furthermore, various cytokines, such as IL-4, IL-17, IL-22, IL-25, and IL-31,^{12,40-42,94} can upregulate the *MAP17* gene,⁹⁵ which further downregulates filaggrin synthesis, ceramide production, and cell-to-cell adhesion, thereby compromising skin barrier function still further. A recent study showed that TSLP levels correlate with xerosis in the SC of patients with AD and

that topical applications of a moisturizer reduced SC TSLP expression.⁸⁶

FILAGGRIN DEFICIENCY PERMITS INCREASED ALLERGEN PENETRATION AND ENHANCES IMMUNE REACTIVITY

Recently generated filaggrin-null mice serve as models for IV without AD.⁹⁶ Here double-allele mutations in pro-*FLG* are responsible for the inherited dysfunctional skin barrier.⁷⁵ Notably, when kept under nonsterile conditions, these mice show accelerated allergen penetration through the SC, as well as an enhanced contact hypersensitivity response.⁹⁶ However, the flaky tail mouse model also had spontaneous inflammation under normal conditions and serves as a model for early AD with filaggrin deficiency. This mouse model also bears a matted mutation (*Tmem79*), which is responsible for the spontaneous dermatitis.⁷⁵ Like the filaggrin-null mouse, the flaky tail mouse also shows increased penetration of allergens, as well as reduced inflammatory threshold levels to skin irritants and allergens.⁹⁷ After sensitization, a further defect in skin barrier function occurs, as indicated by increased transepidermal water loss, suggesting an exacerbation of the barrier deficit by allergic sensitization and inflammation. Thus if the skin barrier is already compromised because of filaggrin deficiency, exposure to penetrating antigens, and/or other stressors, one should activate the inflammasome, proteases, and/or downstream immune mechanisms. For example, mites and cockroaches can secrete and activate SP, thereby causing damage to the barrier along the downstream pathways described above.⁸⁸ Increased SP activity results in increased release of the active forms of IL-1 α and IL-1 β from the compromised corneocytes, which then can initiate inflammation.³¹ The flaky tail mouse also displays increased IL-1 β and TSLP expression and T_H2 polarization.⁸² These alterations in turn activate nuclear factor κ B- and signal transducer and activator of transcription-initiated pathways, increasing *IL6* mRNA levels that likely sustain the inflammatory response in patients with AD.⁹⁸ Accelerated expression of TSLP and IL-6 has also been shown *in vitro* after filaggrin knockdown, again suggesting a link between filaggrin deficiency, T_H2 promotion, and systemic inflammation.^{99,100}

The above murine data, although compelling, have not yet been fully confirmed in human patients with AD. Nonetheless, patients with AD with *FLG* mutations, in contrast to healthy subjects,¹⁰¹ have an increased risk of sensitization to allergens, as well as asthma, rhinitis, and food allergy.¹⁰² For example, *FLG* mutation carriers have a strongly increased risk of AD if exposed to cat, but not dog, allergen at birth.¹⁰³ Although filaggrin deficiency also associates with nickel contact dermatitis, because of the compromised epidermal chelation of nickel, the pathogenic role of nickel in patients with AD, if any, is unclear.^{104,105} Notably, patients with AD with *FLG* mutations display a significant increase in the number of circulating CD4⁺ T_H2 cells compared with those seen in patients with AD with wild-type *FLG*.¹⁰⁶

FILAGGRIN DEFICIENCY PREDISPOSES TO MICROBIAL COLONIZATION AND INVASION

Filaggrin, likely because of its role in SC acidification, seems to protect against colonization with certain microorganisms. Accordingly, acidification of growth medium with exogenous

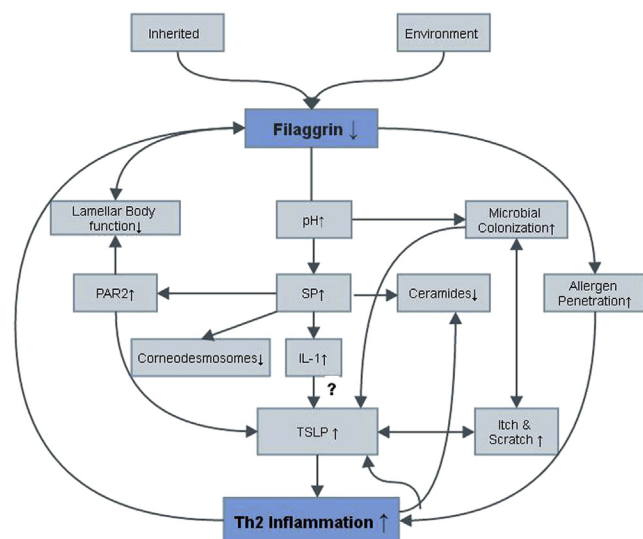


FIG 1. Suggested pathomechanistic pathway linking epidermal filaggrin deficiency to T_H2 inflammation in patients with AD.

UCA or pyrrolidone-5-carboxylic acid reduced growth rates and final cell densities of *Staphylococcus aureus*.¹⁰⁷ Moreover, *S aureus* α -toxin preferentially targets and destroys filaggrin-deficient keratinocytes, whereas normal filaggrin expression increases sphingomyelinase secretion, thereby reducing the number of α -toxin binding sites and risk of colonization.¹⁰⁸ Clinically, this translates into a 7-fold increase in the risk of bacterial infections in patients with *FLG* mutations and AD in comparison with that seen in patients with AD and wild-type *FLG*.¹⁰⁹

Patients with AD and *FLG* mutations also display enhanced risk of eczema herpeticum.¹¹⁰ The role of filaggrin in patients with eczema herpeticum is supported by the observation that IL-25 expression can enhance herpes simplex virus 1 and vaccinia virus replication by downregulating filaggrin expression and also act synergistically with IL-4 and IL-13 to enhance herpes simplex virus 1 replication *in vitro*.^{110,111}

PLACING FILAGGRIN DEFICIENCY IN THE CENTER OF AD PATHOGENESIS

We surmise that AD only develops in those filaggrin-deficient subjects who are exposed to environmental triggers, such as low humidity, high-pH surfactants, pets, pollen, microorganisms, and psychological stress and who can mount an immunologic response to these triggers at the same time. Although it is clear that inherited filaggrin deficiency is not a prerequisite for the development of AD, we believe that acquired reductions of filaggrin and its degradation products in such *FLG* wild-type patients continue to place filaggrin at the very center of AD etiopathogenesis (Fig 1). This view is supported by the observation that homozygous *FLG* mutation carriers who have no filaggrin expression have early-onset AD with a severe and persistent course. Even heterozygous carriers with a less striking barrier abnormality display a significant risk for AD as the strongest genetic risk factor hitherto identified. Filaggrin deficiency results in a barrier deficit that results in a steeper water gradient, even in newborns. Moreover, accelerated water loss, activation of SP and decreased ceramide secretion after

TABLE II. Key observations that place filaggrin at the center of AD etiopathogenesis

- Up to 50% of Irish children with moderate-to-severe AD carry at least 1 mutation in *FLG*.⁸
- Even a slight reduction in the copy numbers of filaggrin from 12 to 10 increases the risk of AD.³⁴
- *FLG* mutations increase the risk of severe AD by all criteria, such as early onset, persistence, infections, hospital visits, and use of medication.¹⁰
- Filaggrin is downregulated in both lesional and nonlesional skin of patients with AD.¹¹
- Filaggrin is downregulated in lesional skin of dark-skinned African subjects, despite the lack of *FLG* mutations.⁵⁵
- Coal tar, a useful therapy for AD, can restore filaggrin expression in *FLG*-haploinsufficient keratinocytes to wild-type levels.¹¹²

alkalinization, and colonization by microbial pathogens further compromise the barrier. Downstream inflammatory-driven downregulation of filaggrin will lead to a further increase in pH, allowing enhanced growth and colonization by pathogenic microorganisms, activation of SP, disruption of the corneodesmosomes, and reduced lamellar body secretion. Yet another vicious cycle is created with a gradual worsening of skin barrier function and a concomitant increase in immune reactivity. Key observations supporting the central role of filaggrin in AD pathogenesis are summarized in Table II.^{8,10,11,34,55,112}

Conclusion and unresolved issues

At present, it is unclear why only a subset (approximately 20% to 30%) of *FLG* mutation carriers have AD and why fewer than 50% of patients with IV have concurrent AD.¹¹³ Therefore future work should continue to identify the molecular basis for the onset of AD, as well as environmental triggers. More comprehensive insights into factors that can downregulate filaggrin are clearly warranted because these might be helpful in preventing barrier impairment and ultimately AD. For example, focused preventive measures can be established, at least in theory, to reduce filaggrin deficiency in at-risk children and patients with AD, potentially involving specific instructions on bathing, clothing, and domestic heating systems. Also, pathophysiologic studies linking filaggrin deficiency with immune dysregulation should be prioritized. Finally, the need for therapies that can increase filaggrin expression in the skin is obvious, and this area should be prioritized. Thus far, functional filaggrin monomers have been successfully linked to cell-penetrating peptides that have reached the stratum granulosum *in vitro* and also restored a normal phenotype in flaky tail mice after topical application.¹¹⁴ Researchers have also identified a candidate drug, JTC801, that promoted *FLG* mRNA and protein expression *in vitro* and after oral administration in NC/Nga mice with AD-like skin disease.¹¹⁵ Therefore The future holds promise that filaggrin restoration can be an important part of AD therapy. In the meantime, traditional therapies should be used to limit inflammation and thereby reduce acquired filaggrin deficiency.

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