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Review

The role of filaggrin in atopic dermatitis and allergic disease

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Key Messages

- Filaggrin is both an important risk factor for atopic dermatitis (AD) and a disease modifier of AD.
- AD patients carrying *FLG* loss-of-function mutations (AD *FLG*) have a distinct clinical and microbiological phenotype.
- Th2 immune skewing causes down-regulation of filaggrin, so that filaggrin is down-regulated in all AD patients, regardless of *FLG* mutation status.
- New sequencing technology allows us to process filaggrin mutations across all ethnic groups and allows identification of previously underreported LoF variants.
- Filaggrin expression is affected by environmental influences such as climate, pollution, water hardness, and the microbiome.

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ABSTRACT

Objective: To provide an overview of filaggrin biology and the role of filaggrin variants in atopic dermatitis (AD) and allergic disease.
Data Sources: We performed a PubMed literature review consisting mainly of studies relating to filaggrin in the last 5 years.
Study Selections: We selected articles that were found in PubMed using the search terms *filaggrin*, *atopic dermatitis*, *skin barrier,* and *atopy.*
Results: Filaggrin plays an important role in the development of AD and allergic disease. Novel methods in measuring filaggrin expression and identifying filaggrin mutations aid in stratifying this patient cohort. We review new insights into understanding the role of filaggrin in AD and allergic disease.
Conclusion: Filaggrin remains a very important player in the pathogenesis of atopic dermatitis and allergic disease. This review looks at recent studies that aid our understanding of this crucial epidermal protein.

Introduction

Filaggrin is a major epidermal protein that has been shown to be a key player in the pathogenesis of atopic dermatitis (AD) and allergic disease. Although filaggrin is a significant genetic risk factor for AD, it is also an important disease modifier in AD. Patients with filaggrin-related AD and asthma have more frequent hospital admissions and long-term medication costs.[1] Atopic dermatitis patients with *FLG* mutations are more frequently affected by reduced health-related quality of life when compared with AD patients with wild-type *FLG*.[2] Although most filaggrin discoveries were originally made in European and Han Chinese populations, advances have been made in determining uncommon loss of function (LoF) variants in *FLG* in several non-European populations. Filaggrin is associated with both the monogenic disease ichthyosis vulgaris (IV) and AD, asthma, and food allergy (FA). Immunological and external environmental mechanisms have influences on filaggrin and contribute to the complex pathogenesis of AD and allergy. We look at the many roles and

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influences on filaggrin and how they contribute to AD and allergic disease.

Filaggrin Biology

Filaggrin is a large (37-kD), histidine-rich protein named after its ability to aggregate keratin intermediate filaments (**Fil**ament **agg**regating Prote**in**).[3] Its larger (>400 kD) precursor profilaggrin is a highly phosphorylated, functionally inactive polymer. Structurally, profilaggrin comprises a S100 calcium binding motif at the N-terminal tandemly linked to 10 to 12 filaggrin monomers and a carboxy-terminal domain.[4,5]

Filaggrin is crucial to the structure and function of the stratum corneum (SC). The SC provides the physical barrier and acts as the first line of host defense against the environment, pathogens, and allergens and is critical for water homeostasis. The epidermis continuously regenerates over approximately 28 days, concluding in the terminal differentiation process known as keratinization or cornification. Migration of keratinocytes from the basal to upper spinous and granular layers marks the onset of cornification.[6] This process results in a tough multilayer cornified envelope made up of 10 to 20 layers of dead cells. The cornified envelope is surrounded by lipids. During differentiation, keratinocytes produce lipids and extrude them into the extracellular space to form lipid-enriched lamellae. The SC has been described as a "bricks and mortar" configuration in which squames are the bricks and the lipid lamellae are the mortar.[7] This continuous lipid matrix is composed mainly of cholesterol, free fatty acids, and ceramides, which regulate the permeability of the epidermal barrier.[8,9] Paracellular proteins, tight junctions, adherens junctions, and desmosomes also provide a permeability barrier and play roles in cell adhesion.[9]

The precursor profilaggrin is expressed late in epidermal differentiation and is stored in keratohyalin granules, which lie in the granular layer.[6] Posttranslational modification of filaggrin is summarized in Figure 1. Profilaggrin is dephosphorylated and cleaved by several endoproteases (including caspase 19)[10] into 10, 11, or 12 filaggrin monomers. Matriptase is an example of an extracellular protease that can alter the expression of filaggrin monomers.[11] Skin aspartic protease/tissue plasminogen activator-inducible aspartic proteinase-like gene/aspartic protease, retroviral-like 1 is a vital protease for this profilaggrin processing.[12] This protease activity is controlled by a number of inhibitors, LETKI encoded by *SPINK5* being the most well characterised. Filaggrin monomers contribute to the mechanical strength of the cytoskeleton by binding to and collapsing keratin filaments into a flattened squame.[13] The keratin filaments are anchored in the SC by corneodesmosomes. Corneodesmosomes, similar to desmosomes, contain cytoplasmic anchoring proteins desmoplakin and plakoglobin, and adhesion molecules, such as desmocollin 1 and desmogelin 1.[14] Filaggrin monomers go through further processing and are deaminated and degraded by proteases. Cysteine/aspartic proteases, bleomycin hydrolase, calpain-1 (C-1), and caspase-14 are important proteases involved in the degradation of filaggrin.[15,16] C-1 also plays a role in maturing corneocytes and profilaggrin processing.[17,18]

Degradation of filaggrin releases a pool of hygroscopic amino acids and derivates of the natural moisturising factor (NMF). Filaggrins' major metabolites are trans-organic acid and pyrrolidone-5-carboxylic acid. These metabolites, together with sodium and chloride ions, urea, and lactate, form the natural moisturising factor.[19] The NMF is vital for skin pH, hydration, UV protection,[20] and the integrity of the epidermal barrier.[21]

Filaggrin Genomics

Profilaggrin is encoded by the *FLG* gene, a large, highly repetitive gene located in the epidermal differentiation complex on chromosome 1q23.3.[3] This is a 3-exon gene with a single exon (exon 3)

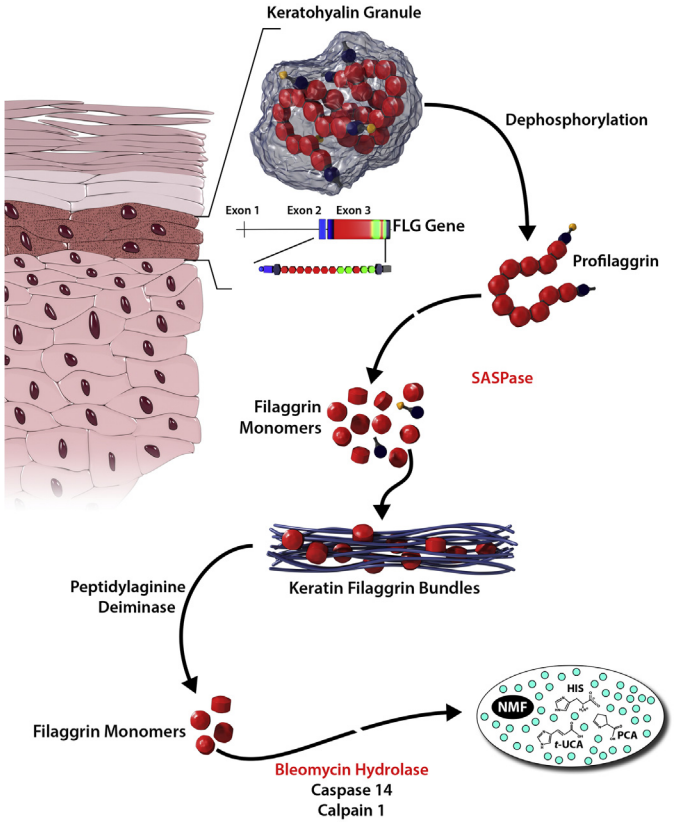

Figure 1. Posttranslational processing of filaggrin. The precursor protein profilaggrin is encoded by exon 3 on the *FLG* gene. Profilaggrin is stored in keratohyalin granules in the stratum granulosum. Profilaggrin is dephosophorylated and cleaved by endoproteases into 10- to 12-filaggrin monomers, which bind to keratin filaments. Filaggrin momomers are deamimated and degraded by proteases, releasing a pool of hygroscopic amino acids and derivatives of the NMF. Adapted from Fleury et al.[122]

coding the entire protein. Diagnostic sequencing of this exon is particularly difficult because of its highly repetitive DNA sequence. The *FLG* gene has intragenic Copy Number Variation (CNV) with alleles encoding either 10, 11, or 12 filaggrin monomers. These variant alleles result in varying levels of filaggrin protein in the epidermis.[22] After excluding *FLG* null mutation carriers, a statistically higher number of copy numbers are seen in controls when compared with AD patients, meaning that low CNV is a risk factor for AD, independent of classic loss-of-function mutations in *FLG*. Linear regression analysis also showed a relationship with urocanic acid concentration (breakdown product of filaggrin) and total copy numbers.[22] *FLG* LoF mutations confer a strong susceptibility to AD.[3,23,24] Loss of function mutations in *FLG* cause complete loss of the expressed protein on that allele. Homozygotes or compound heterozygotes for *FLG* LoF mutations cause complete loss of processed filaggrin, regardless of where the mutation is located.[25] Most studies have looked for the most common LoF variants to report allele frequencies. This can result in the underreporting of the genetic contribution to AD in other ethnic populations in which there is a more diverse range of LoF variants.[26] Unique LoF variants have been demonstrated in different ethnic groups. The 2 most common variants (p.R501X and c.2282del4) in northern Europeans make up 80% of the mutation load.[27] In contrast, East Asian ethnicities show a much more varied mutation range, with fewer prevailing variants.[22,28] A large longitudinal study looked at *FLG* mutations in African American children. Previous studies had been unable to correctly identify *FLG* mutations in this cohort because of technical limitations. This study showed that uncommon *FLG* LoF variants

exist in children of African ancestry and are associated with AD and a more persistent phenotype.[29]

Sanger sequencing the entire *FLG* coding region for large cohorts is the gold standard for classifying patients. However, this is not always feasible or economical. This has been addressed with the development of fluidigm microfluidics technology and next-generation sequencing.[30] This is a reliable, cost-effective polymerase chain reaction–based method for analyzing the entire coding region of *FLG*. Use of this technology to fully resequence cohorts of the Indian, Malay, and Chinese patients with AD in the Singapore population identified many unreported LoF mutations and confirmed the variety of *FLG* variants that exist between different ethnicities. This method is considerably (×10) cheaper than standard exome sequencing and gives a more accurate estimate of the genetic contribution from deleterious alleles and CNVs in AD.[30]

Regional Variation in Filaggrin Expression

Regarding sites of AD lesions, it is well known that infantile AD has common sites of predilection. Atopic dermatitis characteristically affects the face in infants, sparing the nasal tip in almost all cases.[31] This suggests that there are structural and functional differences in the skin at different sites. Differences between the structure of adult and childhood skin have been observed.[32,33] Corneocytes in infants are smaller and show variation in size, reflecting the high epidermal turnover rate.[34] Filaggrin plays a crucial role in the structural integrity of the SC and recently its' role in the site predilection of infantile AD has been reviewed.[35] The NMF levels at different anatomical sites of 129 children were studied. The NMF levels were low at birth and rose significantly over the first 4 weeks of life. The NMF levels of the cheek skin in particular were slow to rise compared with other sites (eg, nasal tip, elbow). The cheek is often the original site of AD in this infantile cohort. The elbow, an unusual site for AD in these patients, reached steady states of NMF early compared with the cheek. The elbow is a classic site for AD in later childhood years. This could be caused by secondary effects such as the microbiome of this region; age strongly affects the diversity of host commensals.[36] The activity of SC proteases (BH, C-1, and plasmin) important in filaggrin degradation were also studied. At 1 month of age, these proteases were shown to be elevated in the cheek skin but not in the elbow skin.

Measuring Filaggrin Protein Expression In Vivo

The most widely used methods of measuring filaggrin expression in vivo are Raman microspectroscopy (RMS) and stratum corneum tape stripping technique, followed by high-performance liquid chromatography (TS/HPLC), which collects SC layer samples using a minimally invasive tape stripping technique involving adhesive tapes. The filaggrin degradation products are extracted by acid or base and identified using HPLC.[37] Raman spectroscopy is an optical method of measuring filaggrin expression in vivo. The underlying process is based on the inelastic scattering of monochromatic light when the photon frequency, normally from a laser source, changes as it comes into contact with a sample. This determines in real time the levels of several filaggrin degradation products at different levels of the SC.[38,39] This results in characteristic Raman spectra, which are noninvasive molecular measurements of filaggrin degradation products within the SC.[40]

Koppes et al[41] compared both RMS and HPLC. They showed a strong correlation between the 2 methods, despite the methods measuring different NMF components. This supported previous data that showed that different filaggrin degradation products are highly correlated.[42] Both methods showed good reproducibility and robustness. Thus, the choice between these methods will likely be affected by factors such as cost, time constraints, expertise, and accessibility. Raman microspectroscopy can provide rapid comprehensive data on the profile of the filaggrin degradation products across the SC. However, this method is more costly and less accessible than HPLC. Tape stripping allows quick and easy collection of samples that can be stored for a longer time. However, unlike RMS, it requires further processing for HPLC analysis, prolonging the time to results.[41]

O'Regan et al[13] looked at the relationship between Raman NMF signatures of the stratum corneum and *FLG* mutation status in patients with moderate-to-severe atopic dermatitis.

They found that Raman NMF could differentiate between *AD FLG* and *AD non-FLG* as well as discriminate from heterozygous mutations with high specificity. Thus, Raman NMF values can be used to predict *FLG* genotypes in AD patients. A tyrosine signal in patients with *FLG* mutations carried a strong predictive value of *FLG* null mutations. This was proposed as a potential biomarker for this diagnosis. Filaggrin lacks tyrosine; profilaggrin, however, possesses tryrosine-rich motifs at its carboxy terminal domain and linker segments.[43-45] The strong presence of tyrosine in *FLG* null carriers is proposed to be secondary to altered processing pathways such as the proteolysis of profilaggrin.

Other forms of measuring filaggrin expression also exist. Hwang et al[46] used PNA (peptide nucleic acid) probes to accurately and rapidly detect *FLG* mutations. The PNAs are artificial DNA analogues that pair with their complementary DNA targets. The PNA-DNA hybridization is more sensitive than DNA-DNA hybridization to base mismatches. This results in a distinct difference in the melting temperatures between perfectly matched and mismatched DNA sequences. Thus, through this PNA probe-based fluorescence melting curve analysis they were able to detect both heterozygous and homozygous *FLG* mutations with precision.[46]

Filaggrin Disease Associations

Monogenic Disease

Ichthosis vulgaris (IV) is the most common disorder of keratinization and 1 of the most prevalent single-gene disorders. It is caused by LoF mutations in *FLG*.[47] Clinically, hyperlinearity of the palms and soles, dry skin, and keratosis pilaris are seen. It is inherited in an autosomal semidominant pattern; patients with a single mutation have very mild or no disease, whereas those with 2 mutations have a more severe phenotype.[47,48] Heterozygous *FLG* mutations are thought to be associated with intermediate hyperlinearity, and *FLG*-related AD has been associated with hyperlinearity.[47,49-51] O'Regan et al[13] found that in patients with moderate-to-severe AD, NMF and *FLG* status correlated with hyperlinearity.

Filaggrin in Atopic Dermatitis

The most significant genetic risk factor for AD is LoF mutations of *FLG*.[52] The odds ratio regarding LoF mutations in *FLG* and AD has been estimated by meta-analysis to range from 3.12 to 4.78.[52,53] Maternal *FLG* mutations have been shown to increase the risk of AD independent of mutation inheritance.[54] Atopic dermatitis is not a single homogeneous disease but rather a variety of different disease phenotypes. Recent studies have classified different endotypes according to several factors. These include age, chronicity, ethnicity, immunoglobulin E (IgE) levels, and *FLG* mutation status.[55] Atopic dermatitis associated with *FLG* mutations has a distinct phenotype (AD FLG). Clinical features of this endotype include palmer hyperlinearity, increased risk of asthma, eczema herpeticum increased severity, and allergic sensitization (Fig 2). This endotype is also associated with a more persistent clinical course. Paternoster et al[56] have demonstrated 6 separate subphenotypes of

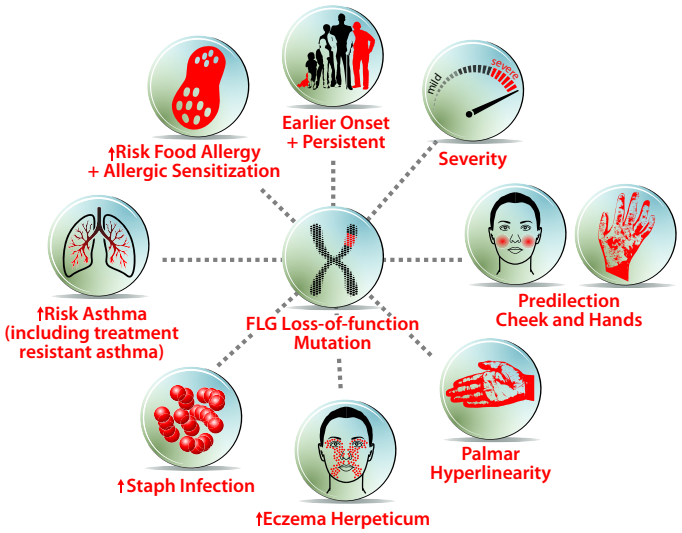

Figure 2. The AD *FLG* phenotype; earlier onset and persistent disease, increased severity, predilection to the cheeks and hands, palmar hyperlinearity, increased risk of eczema herpeticum and staphylococcal infection, increased risk of asthma (including treatment-resistant asthma), and increased risk of food allergy as well as allergic sensitization. FLG, gene-encoding filaggrin; AD, atopic dermatitis; SA, *Staphylococcal aureus,* AD FLG, atopic dermatitis associated with loss of function mutations in filaggrin; LoF, loss of function; FA, food allergy; SC, stratum corneum; FFA, free fatty acids; NMF, natural moisturizing factor; CNV, copy number variation; RMS, Raman microspectroscopy; HPLC, high-performance liquid chromatography; TEWL, transepidermal water loss.

AD in childhood, each with different disease trajectories. The *FLG* null mutations were often associated with all subphenotypes and most strongly associated with the early-onset persistent disease subgroup.[56] The most persistent endotypes were associated with *FLG* null mutations and had the greatest risk of asthma, raised IgE levels, and a parental history of atopy. Atopic dermatitis patients with persistent disease are more likely to have *FLG* mutations[57] and carry a higher risk of skin infections with herpes virus.[58] Atopic dermatitis *FLG* are associated with a higher risk of multiple allergies.[50,53,57] and asthma[57,59] (Fig 2). Palmer hyperlinearity was also associated with sensitization to egg.[60] The *FLG* mutations also denote a more severe course in alopecia areata,[61] and they also have been associated with increased lifetime prevalence of hand and foot dermatitis in patients with a diagnosis of AD.[62]

Asthma, Allergic Rhinitis, and Food Allergy

FLG mutations are a risk factor for the development of asthma, allergic rhinitis, and food allergy. The *FLG* LoF mutations in patients with a history of AD are associated with an increased risk of asthma ranging from 1.48 to 1.79.[57,63] Filaggrin is not found in respiratory epithelia.[64] The fact that AD has an earlier age of onset than asthma and AR has led to a causal relationship being proposed. Atopic dermatitis has therefore been seen as the entry point for consequent allergic disease.[65]

The atopic march suggests a progression of AD to allergic disease,[65-67] although many patterns and temporal relationships between these conditions exist. Atopic dermatitis is often the first manifestation, because characteristics of AD, including a skin barrier defect and inflammation, may facilitate development of subsequent comorbidities. Previous studies have shown that AD increases risk of asthma if the patient had a history of allergy to food or other inhalants.[68] This observation could propose that FA could correlate with AD severity or represent an AD endotype that is more inclined to have allergic comorbidities.[66,69] Loss of function mutations in *FLG* predispose to peanut allergy, excluding for patients with AD.[70] In addition to this, high environmental exposure to peanut allergen increases peanut allergy risk in both *FLG* LoF mutation carriers[71] and patients with AD.[72] More recently, a significant association has been found with *FLG* LoF mutations and polysensitivity in patients with a history of AD.[73] Filaggrin and ceramide content in the nonlesional skin of AD FA+ was substantially lower when compared with AD FA− and NA skin.[74] The relationship between FA and AD may primarily involve an inferior epidermal barrier. Ziyab et al[75] showed that the combined effects of 'preceding allergic sensitisation and *FLG* variants' and 'preceding atopic dermatitis and *FLG* variants 'increased the risk of subsequent asthma by 4.93 and 3.33-fold respectively.[75] Neither of these effects showed a significant correlation with increased risk of rhinitis, suggesting that the mechanisms in which *FLG* variants predispose to asthma and rhinitis may be different.

Filaggrin Interactions with Dysregulated Immune Responses in Atopic Dermatitis

Only 10% to 40% of AD patients have LoF mutations in *FLG*,[3,24] and most patients with AD outgrow their disease.[76,77] In addition, those with bi-allelic *FLG* mutations do not always have a diagnosis of AD.[78] This suggests that there must be other mechanisms at play that modulate the expression of filaggrin and the integrity of the epidermal barrier. In AD, there is overexpression of type 2 cytokines, especially IL-4 and IL-13, which down-regulate epidermal barrier genes.[79,80] Howell et al[81] looked at normal primary keratinocytes in the presence and absence of IL-4 and Il-13 to determine whether this modified filaggrin expression. They showed that filaggrin expression was induced by differentiation alone; however, it was significantly reduced with exposure to IL-4 and IL-13. Their findings strongly suggest acquired filaggrin deficiency in patients with AD secondary to type 2 cytokines. Previous evidence suggests a skin barrier defect of both lesional and nonlesional skin in AD patients.[82-84] Transepidermal water loss (TEWL), a marker of barrier disruption and epidermal water loss, has been shown to be increased in nonlesional skin in AD.[85-87] O'Regan et al[40] found that TEWL in patients with moderate-to-severe AD does not discriminate between *FLG* genotype. The secondary immunologic response appears to drive increased TEWL in these patients. In vivo, Kezic et al[42] found significantly decreased levels of NMF in the nonlesional skin of AD patients without *FLG* mutations. This suggests a systemic down-regulation of filaggrin at play. They also found that AD severity impacted NMF levels, implying that a reduction of NMF is a broad feature of AD. The up-regulation of filaggrin expression could therefore be a potential therapeutic pathway for all patients with AD regardless of mutation status. The NMF levels could be used as an investigative tool to measure response to treatment.[42]

The *FLG* null mutations increase levels of circulating thymic-derived Tregs and limit the expansion of both memory and effector T cells, further promoting Treg abnormalities seen in AD. *FLG* null mutations also heightened the immune imbalance between Th-1−, Th-2−, and Th-17−like Tregs in AD.[88] Leitch et al[89] showed that *FLG* null mutation carriers have more mature Langerhans cells in nonlesional skin irrespective of whether they have a diagnosis of AD. Also, filaggrin degradation products, including the cis-isomer of urocanic acid, were shown to down-regulate the expression of dendritic cells and increase the production of regulatory T-cells.[89] Filaggrin-deficient mice were shown to have higher levels of peripheral Th17 cells than their wild-type controls. This observation was present in adult ft/ft mice but not in 2-week-old ft/ft mice, indicating that this must be an acquired phenomenon.[90]

Exposomal Interactions with Filaggrin

The exposome refers to the sum of external influences to which an individual is exposed.[91] This encompasses multiple personal and

environmental factors.[92] There appears to be a complex interplay between a genetically susceptible barrier, further external insults on this barrier, and a dysfunctional immune system, resulting in increased itch and scratch. This is a perpetuating cycle of further barrier disruption and vulnerability to external exposures.[86] Regarding environment, it is well known that there is a geographical variation in the prevalence of AD. In the past, this was seen in urbanized areas of economic development. The ISAAC Phase Three found that disease burden in these areas has plateaued over time. Instead, the increase has been seen in lower-income settings, which had a low prevalence previously.[93] This supports the important role of exposomal influence. These findings could be explained by the fact that those who were genetically predisposed to AD, such as *FLG* mutations, did not face any new further exposomal insults than they had previously been exposed to. Whereas more exposomal change through increased urbanization has driven numbers of AD in the previous low-prevalence areas.

The local climate exerts influence on AD, with ultraviolet (UV) radiation, temperature, and humidity contributing to AD flares and varied regional prevalence. Low use of central heating, high temperatures, and relative humidity drive down the frequency of AD.[94] Filaggrin and its breakdown products appear to be central to this phenomenon. Filaggrin expression is decreased by low humidity, whereas filaggrin proteolysis is concurrently increased.[95-97] Trans-urocanic acid (a filaggrin breakdown product) is transformed to the immunosuppressive cis-urocanic acid by UV light. This helps dampen down the immune response during AD flares.[98,99] These 2 mechanisms inhibit suppression of filaggrin as well as accelerate its breakdown. Hence those with mutations in filaggrin are particularly vulnerable to these processes.[100]

As stated, AD is increased in areas of urbanisation. Exposure to airborne pollutants such as traffic-related pollutants, benzene, nitrogen compounds, and antenatal and postnatal tobacco smoke exposure have been shown to increase the risk of developing AD and contribute to flares.[101-104] Epigenetic changes in utero as well as direct damage to the stratum corneum by reactive oxygen species has been proposed as the underlying mechanism.[105] Urban particulate matter has also been shown to decrease filaggrin expression by inducing cyclooxygenase 2/prostaglandin E_2.[106] This epigenetic immune priming allows type 2 cytokines to drive inflammation and itch, down-regulating filaggrin and perpetuating this damaging cycle.[107] Increased water hardness and detergent use are other factors that have been shown to increase risk of AD. They do this by raising the surface pH, which diminishes NMF and increases protease activity.[108] Sodium lauryl sulfate (SLS) is a substance commonly found in washing products and is known to cause irritation and increased TEWL particularly those with *FLG* mutations.[109] It is believed sodium lauryl sulfate does this by inhibiting the expression of profilaggrin.[110] The water gradient in the SC controls filaggrin breakdown.[95] Filaggrin expression was decreased by 50% after 24 hours of occluded water exposure. Water hardness had the most detrimental effect on the epidermal barrier.[111] High calcium carbonate is usually associated with a raised pH, which can prematurely activate serine proteases that break down corneodesmosomes.[112]

The microbiome is a key player in the conversation between the environment and the host and is susceptible to external insults.[113] Mode of delivery influences the neonatal microbiome. Those delivered vaginally have microbiomes dominated by lactobacilli, in contrast to infants born by caesarean section, who are colonized by maternal commensals.[114] A healthy cutaneous microbiome consists of a diverse range of microorganisms.[115]

The postnatal development of this varied microbiome begins with rapid colonization of commensals such as *Staphylococcus epidermidis*. Treg cells are up-regulated to allow epidermal tolerance of these commensals to develop. Thus, extensive interaction occurs between the microbiome and the immune system to preserve a healthy state.[114,116] This potential to develop tolerance declines with age [67] and explains why antibiotic use in early life is associated with increased risk of AD.[117,118]

Decreased diversity and expansion of pathogenic species such as staphylococci have been found in both lesional and nonlesional skin in AD.[119] *SA* is particularly pathogenic, colonizing over 90% of AD patients.[120] *SA* secretes and displays virulence factors and superantigens that induce type 2 cytokines to drive inflammation and itch and further damage the epidermal barrier.[121] Deficiency in filaggrin and thus NMF allows *SA* to adhere to the epidermis. *SA* binds via its clumping factor B, whose expression is up-regulated by decreased levels of NMF.[122,123] Commensal coagulase-negative staphylococci produce bactericidal peptides that inhibit *SA* biofilm formation. Commensal numbers are reduced in the early stages of AD.[36,124,125] Differences in the microbiome composition in relation to filaggrin status have recently come to light. These were found in nonlesional skin and affected the beta diversity with increased numbers of *Staphylococcus caprae* in relation to filaggrin status.[126] Increased *SA* colonization in AD *FLG* patients has also been shown.[127]

Given the accessibility of the cutaneous microbiome, it is a prime target for therapeutic manipulation, which aims to increase microbial diversity. Increased microbial diversity is seen during recovery of AD flares[119] and after standard AD treatments.[128,129]

Recently, new theory suggests that increased contact with nature denotes increased microbial diversity of the skin. Pet ownership, particularly dog ownership, has been shown to be associated with the development of AD.[67,130,131]

Therapeutic Approaches and Influence of Filaggrin Mutations on Response to Treatment

As stated previously, the classical atopic march is thought to be initiated by a defective skin barrier, which allows a skewed type 2 response and creates a subsequent pro inflammatory climate.[132] Thus, early recovery of the skin barrier may be of vital importance in preventing ensuing allergic disease.[133] Early use of an emollient could be a simple and cost-effective strategy[134-136]; however, recent studies have not yet shown a clear benefit. Occlusion with petrolatum has been shown to alter the structure of the epidermis and increase expression of terminal differentiation proteins, including filaggrin.[137] Stout et al[138] showed success with the topical application of a functional filaggrin monomer covalently linked to a cell-penetrating peptide in murine models. It was taken up by the human epidermal model and helped restore the normal phenotype.[138] Because filaggrin is an entirely intracellular protein, expressed at a crucial time and location during terminal epidermal differentiation, replacement via topical applications remains a challenge. We have reviewed the technical difficulties elsewhere.[139]

FLG mutation status is also important regarding treatment effectiveness. Chang et al[140] looked at the number of *FLG* LoF phenotype and treatment use in a pediatric population. They found that treatment use and likely effectiveness were influenced by genetic variation. This variation was limited to 2 *FLG* LoF alleles and TSLPrs1898671 homozygotes (thymic stromal lymphopoietin), with no significant difference seen between the wild-type and heterozygous patients in most of the outcomes they studied.[140]

Conclusion

To conclude, with new sequencing technology, it is now possible to process *FLG* mutations on a deeper and wider scale across all ethnicities. Previously uncommon LoF variants in other ethnic populations have been underreported. Filaggrin is well known to play a central part in the pathogenesis of AD. More is now known of the distinct AD *FLG* phenotype. Sites of predilection, allergy, asthma, severity, microbial influence, and persistent course are a few things

that distinguish these patients. Reliable and accurate methods of measuring filaggrin expression can be used to assess post-translational filaggrin expression. There are many internal and external influences on filaggrin expression. The immune system is capable of causing an acquired filaggrin deficiency, and conversely, *FLG* null mutations drive immune responses. Externally, exposomal influences such as climate change, pollution, water hardness, and the microbiome all have significant effects on filaggrin. *FLG* gene testing remains an investigational tool rather than a bedside test. As many novel systemic therapies proceed through clinical trials, we will learn more about the effects of carrying a *FLG* mutation on response to drug therapy.

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