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Catherine Drislane, MB, Alan D. Irvine, MD DSc

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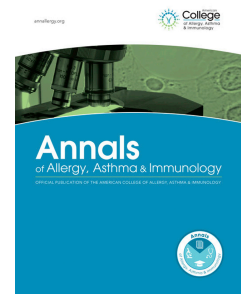
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# ***The Role of Filaggrin in Atopic Dermatitis and Allergic Disease***

Catherine Drislane MB and Alan D. Irvine MD DSc

Catherine Drislane MB  
Clinical Medicine, Trinity College Dublin  
[drislac@tcd.ie](mailto:drislac@tcd.ie)

Alan D. Irvine MD DSc  
Department of Paediatric Dermatology, Our Lady's Children's Hospital Crumlin, Dublin,  
National Children's Research Centre, Crumlin and Clinical Medicine, Trinity College  
Dublin, Ireland  
[irvinea@tcd.ie](mailto:irvinea@tcd.ie)

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Atopic dermatitis, ichthyosis, skin barrier, allergy, genetics, filaggrin, FLG.

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## **Table of Abbreviations**

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 Moisturising Factor  
CNV: Copy Number Variation  
RMS: Raman microspectroscopy  
HPLC: high performance liquid chromatography  
TEWL: Transepidermal water loss

## 1 Introduction

2  
3 Filaggrin is a major epidermal protein that has been shown to be a key player in the  
4 pathogenesis of AD and allergic disease. While filaggrin is a significant **genetic risk factor** for  
5 AD, it is also an important **disease modifier** in AD. Patients with filaggrin related AD and asthma  
6 have more frequent hospital admissions and long-term medication costs[1]. Atopic dermatitis  
7 patients with *FLG* mutations are more frequently affected by reduced health related quality of  
8 life when compared to AD patients with wild-type *FLG*[2]. While most filaggrin discoveries were  
9 originally made in European and Han Chinese populations, advances have been made in  
10 determining uncommon LOF variants in filaggrin in several non-European populations. Filaggrin  
11 is associated with both the monogenic disease, ichthyosis vulgaris (IV) as well as atopic  
12 dermatitis (AD), asthma and food allergy (FA). Immunological and external environmental  
13 mechanisms have influences on filaggrin and contribute to the complex pathogenesis of AD and  
14 allergy. In this review we look at the many roles and influences on filaggrin and how they  
15 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 (**Filament aggregating Protein**) [3]. Its larger (> 400kD) precursor profilaggrin is a highly phosphorylated, functionally inactive polymer. Structurally, profilaggrin comprises a S100 calcium binding motif at the N-terminal tandemly linked to 10-12 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 defence 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 10-20 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 comprises mainly of cholesterol, FFA 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]. Post translational modification of filaggrin is summarised 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]. SASpase/Taps/ASPRV1 ( skin aspartic protease/TPA-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; for example desmocollin 1 and desmoglein 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 derivatives of the natural moisturising factor (NMF). Filaggrins' major metabolites are trans-organic acid (trans-UCA) and pyrrolidone-5-carboxylic acid (PCA). These metabolites, together with sodium and chloride

ions, urea, and lactate, form the natural moisturising factor[19]. TNMF is vital for skin pH, hydration, UV protection[20] as well as 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 three exon gene with a single exon (exon 3) coding the entire protein. Diagnostic sequencing of this exon is particularly difficult due to 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, it has been shown that there is a statistically higher number of copy numbers in controls when compared to atopic dermatitis (AD) patients, meaning 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]. Filaggrin loss-of-function (LoF) mutations confer a strong susceptibility to AD[3, 23, 24] *LOF 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]. To date, the majority of studies have looked for the most common LoF variants to report allele frequencies. This can result in the under reporting of the genetic contribution to AD in other ethnic populations where there is a more diverse range of LoF variants[26]. Unique LoF variants have been demonstrated in different ethnic groups. The two most common variants ( p.R501X and

c.2282del4) in norther 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. Previously studies had been unable to correctly identify *FLG* mutations in this cohort due to 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 (NGS)[30]. This is a reliable, cost effective PCR based method for analysing the entire coding region of *FLG*. Utilization 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 exists between different ethnicities. This method is considerably (x10) 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. AD 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. NMF levels were low at birth and rose significantly over the first 4 weeks of life. NMF levels of the cheek skin in particular, were slow to rise compared to other sites (e.g. nasal tip, elbow). Interestingly, 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 to the cheek. The elbow is a classic site for AD in later childhood years. This could be due to secondary effects such as the microbiome of this region as we know 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 one 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). TS/HPLC collects SC layer samples using a minimally invasive tape stripping technique using 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 compared both RMS and HPLC [41]. They showed a strong correlation between the two 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. RMS 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 which 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 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 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. Interestingly, 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 used PNA (peptide nucleic acid) probes to accurately and rapidly detect *FLG* mutations. 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**

Ichthyosis vulgaris (IV) is the most common disorder of keratinization and one of the most prevalent single gene disorders. IV is caused by loss of function mutations in *FLG*[47]. Clinically, there is hyperlinearity of the palms and soles, dry skin and keratosis pilaris. It is inherited in an autosomal semi-dominant pattern: patients with a single mutation have very mild or no

disease, while 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]. Regan et al found that in patients with moderate-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 filaggrin mutations have been shown to increase the risk of AD independent of mutation inheritance[54]. AD 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, IgE levels and *FLG* mutation status[55]. AD 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 sensitisation (**Figure 2**). This endotype is also associated with a more persistent clinical course. Paternoster et al have demonstrated 6 separate subphenotypes of AD in childhood, each with different disease trajectories. *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. AD patients with persistent disease are more likely to have *FLG* mutations [57]

and carry a higher risk of skin infections with herpes virus[58]. AD *FLG* are associated with a higher risk of multiple allergies [50, 53, 57] and asthma [57, 59] (**Figure 2**). Palmer hyperlinearity was also associated with sensitisation to egg [60] *FLG* mutations also denote a more severe course in alopecia areata[61] and have also 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**

Filaggrin mutations are a risk factor for the development of asthma, allergic rhinitis and food allergy. *FLG* LoF mutations in patients with a history of AD are associated with an increased risk of asthma ranging from 1.48-1.79 [57, 63] Filaggrin is not found in respiratory epithelia [64]. The fact that AD has an earlier age of onset than asthma and AR a causal relationship has been proposed. AD 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 exists. AD is often the first manifestation as characteristics of AD including a skin barrier defect and inflammation may facilitate development of subsequent comorbidities. Previous studies have showed that AD increases risk of asthma if patients 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 which is more inclined to have allergic comorbidities[66, 69]. LoF 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 AD[72]. More recently, a significant association has been found with filaggrin loss of function mutations and polysensitivity in patients with a history of atopic dermatitis [73]. Filaggrin and ceramide content in the non-lesional skin of AD FA + was substantially lower when compared to AD FA – and NA skin[74]. The relationship between FA and AD may primarily involve an inferior epidermal barrier. Ziyab et al 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 10-40% 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 downregulate 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 filaggrin expression was induced by differentiation alone however it was significantly

reduced with exposure to IL-4 and IL-13. Their findings strongly suggest that there is acquired filaggrin deficiency in patients with AD secondary to type 2 cytokines. Previous evidence suggests that there is a skin barrier defect of both lesional and non-lesional skin in AD patients [82-84]. TEWL, a marker of barrier disruption and epidermal water loss, has been shown to be increased in non-lesional skin in AD [85-87]. O'Regan et al 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 found significantly decreased levels of NMF in the non-lesional skin of AD patients without *FLG* mutations. This suggest a systemic downregulation 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 upregulation of *filaggrin* expression could therefore be a potential therapeutic pathway for all patients with AD regardless of mutation status. Leading on from this, NMF levels could be used as an investigatory tool to measure response to treatment[42].

*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 showed that *FLG* null mutation carriers have more mature Langerhans cells in non-lesional skin irrespective of whether they have a diagnosis of AD. Also, filaggrin degradation products, including the cis-isomer of UCA, were shown to downregulate 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 two-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 urbanised 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, eg *FLG* mutations, did not face any new further exposomal insults than they had previously been exposed to. Whereas more exposomal change through increased urbanisation has driven numbers of AD in the previous low prevalence areas.

The local climate exerts influence on AD with 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 while 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 two 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, 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 COX2/PGE2[106] This epigenetic immune priming allows type 2 cytokines to drive inflammation and itch, downregulating 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 SLS 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 colonised by maternal commensals. [114] A healthy cutaneous microbiome consists of a diverse range of microorganisms.[115] The post-natal development of this varied microbiome begins with rapid colonisation of commensals such as staph epidermidis. Treg cells are upregulated to allow epidermal tolerance of these commensals to develop. Thus there is extensive interaction 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 non-lesional skin in AD. [119] SA is particularly pathogenic colonising over 90% of AD patients. [120] SA secretes and displays virulence factors and superantigens that induce type 2 cells 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 upregulated by decreased levels of NMF.

[122, 123] Commensal coagulase negative Staph 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 non-lesional skin and affected the beta diversity with increased numbers of *S Caprae* in relation to filaggrin status[126]. Increased *SA* colonisation 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 following standard AD treatments. [128, 129] Recently, new theory suggests that increased contact with nature denotes increased microbial diversity of the skin. Pet owners, 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 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 showed success with the topical application of a functional filaggrin monomer covalently linked to a cell penetrating peptide ( RMR) in murine models. It was taken up by the human epidermal model and helped restore the normal phenotype.[138] As 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].

Filaggrin mutation status is also important regarding treatment effectiveness. Chang et al looked at the number of *FLG* LoF phenotype and treatment use in a paediatric 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 wildtype and heterozygous patients in the majority of the outcomes they studied[140].

## Conclusion

To conclude, with new sequencing technology it is now possible to process filaggrin mutations on a deeper and wider scale across all ethnicities. Previously uncommon LoF variants in other ethnic populations have been underreported. It is well known that filaggrin plays 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.*

1. Soares, P., et al., *Individuals with filaggrin-related eczema and asthma have increased long-term medication and hospital admission costs*. Br J Dermatol, 2018. **179**(3): p. 717-723.
2. Heede, N.G., et al., *Health-related quality of life in adult dermatitis patients stratified by filaggrin genotype*. Contact Dermatitis, 2017. **76**(3): p. 167-177.
3. Irvine, A.D., W.H. McLean, and D.Y. Leung, *Filaggrin mutations associated with skin and allergic diseases*. N Engl J Med, 2011. **365**(14): p. 1315-27.
4. Fleckman, P., B.A. Dale, and K.A. Holbrook, *Profilaggrin, a high-molecular-weight precursor of filaggrin in human epidermis and cultured keratinocytes*. J Invest Dermatol, 1985. **85**(6): p. 507-12.
5. Markova, N.G., et al., *Profilaggrin is a major epidermal calcium-binding protein*. Mol Cell Biol, 1993. **13**(1): p. 613-25.
6. Sandilands, A., et al., *Filaggrin in the frontline: role in skin barrier function and disease*. J Cell Sci, 2009. **122**(Pt 9): p. 1285-94.
7. Nemes, Z. and P.M. Steinert, *Bricks and mortar of the epidermal barrier*. Exp Mol Med, 1999. **31**(1): p. 5-19.
8. Candi, E., R. Schmidt, and G. Melino, *The cornified envelope: a model of cell death in the skin*. Nat Rev Mol Cell Biol, 2005. **6**(4): p. 328-40.
9. Kim, B.E. and D.Y.M. Leung, *Significance of Skin Barrier Dysfunction in Atopic Dermatitis*. Allergy Asthma Immunol Res, 2018. **10**(3): p. 207-215.
10. Denecker, G., et al., *Caspase-14 protects against epidermal UVB photodamage and water loss*. Nat Cell Biol, 2007. **9**(6): p. 666-74.
11. Tan, S.P., et al., *Protease-antiprotease imbalance may be linked to potential defects in profilaggrin proteolysis in atopic dermatitis*. Br J Dermatol, 2012. **166**(5): p. 1137-40.
12. Matsui, T., et al., *SASPase regulates stratum corneum hydration through profilaggrin-to-filaggrin processing*. EMBO Mol Med, 2011. **3**(6): p. 320-33.
13. O'Regan, G.M., et al., *Filaggrin in atopic dermatitis*. Journal of Allergy and Clinical Immunology, 2009. **124**(3): p. R2-R6.
14. Matsui, T. and M. Amagai, *Dissecting the formation, structure and barrier function of the stratum corneum*. Int Immunol, 2015. **27**(6): p. 269-80.
15. Kamata, Y., et al., *Quantification of neutral cysteine protease bleomycin hydrolase and its localization in rat tissues*. J Biochem, 2007. **141**(1): p. 69-76.
16. Kamata, Y., et al., *Neutral cysteine protease bleomycin hydrolase is essential for the breakdown of deiminated filaggrin into amino acids*. J Biol Chem, 2009. **284**(19): p. 12829-36.
17. Yamazaki, M., et al., *Cytoplasmic processing of human profilaggrin by active mu-calpain*. Biochem Biophys Res Commun, 1997. **235**(3): p. 652-6.
18. Hsu, C.Y., et al., *Deimination of human filaggrin-2 promotes its proteolysis by calpain 1*. J Biol Chem, 2011. **286**(26): p. 23222-33.
19. Brown, S.J. and W.H. McLean, *One remarkable molecule: filaggrin*. J Invest Dermatol, 2012. **132**(3 Pt 2): p. 751-62.

20. Barresi, C., et al., *Increased sensitivity of histidinemic mice to UVB radiation suggests a crucial role of endogenous urocanic acid in photoprotection*. J Invest Dermatol, 2011. **131**(1): p. 188-94.
21. Rawlings, A.V. and C.R. Harding, *Moisturization and skin barrier function*. Dermatol Ther, 2004. **17 Suppl 1**: p. 43-8.
22. Brown, S.J., et al., *Intragenic copy number variation within filaggrin contributes to the risk of atopic dermatitis with a dose-dependent effect*. J Invest Dermatol, 2012. **132**(1): p. 98-104.
23. Rogers, A.J., et al., *Filaggrin mutations confer susceptibility to atopic dermatitis but not to asthma*. J Allergy Clin Immunol, 2007. **120**(6): p. 1332-7.
24. Palmer, C.N., et al., *Common loss-of-function variants of the epidermal barrier protein filaggrin are a major predisposing factor for atopic dermatitis*. Nat Genet, 2006. **38**(4): p. 441-6.
25. Sandilands, A., et al., *Prevalent and Rare Mutations in the Gene Encoding Filaggrin Cause Ichthyosis Vulgaris and Predispose Individuals to Atopic Dermatitis*. Journal of Investigative Dermatology, 2006. **126**(8): p. 1770-1775.
26. Clark, A.G., et al., *Ascertainment bias in studies of human genome-wide polymorphism*. Genome Res, 2005. **15**(11): p. 1496-502.
27. Sandilands, A., et al., *Comprehensive analysis of the gene encoding filaggrin uncovers prevalent and rare mutations in ichthyosis vulgaris and atopic eczema*. Nat Genet, 2007. **39**(5): p. 650-4.
28. Chen, H., et al., *Wide spectrum of filaggrin-null mutations in atopic dermatitis highlights differences between Singaporean Chinese and European populations*. Br J Dermatol, 2011. **165**(1): p. 106-14.
29. Margolis, D.J., et al., *Uncommon Filaggrin Variants Are Associated with Persistent Atopic Dermatitis in African Americans*. J Invest Dermatol, 2018. **138**(7): p. 1501-1506.
30. Wong, X., et al., *Array-based sequencing of filaggrin gene for comprehensive detection of disease-associated variants*. J Allergy Clin Immunol, 2018. **141**(2): p. 814-816.
31. Yamamoto, K., *Studies on autosensitization dermatitis*. Keio J Med, 1961. **10**: p. 59-78.
32. Ludriksone, L., et al., *Skin barrier function in infancy: a systematic review*. Arch Dermatol Res, 2014. **306**(7): p. 591-9.
33. Stamatas, G.N., et al., *Infant skin physiology and development during the first years of life: a review of recent findings based on in vivo studies*. Int J Cosmet Sci, 2011. **33**(1): p. 17-24.
34. Stamatas, G.N., et al., *Infant skin microstructure assessed in vivo differs from adult skin in organization and at the cellular level*. Pediatr Dermatol, 2010. **27**(2): p. 125-31.
35. McAleer, M.A., et al., *Early-life regional and temporal variation in filaggrin-derived natural moisturizing factor, filaggrin-processing enzyme activity, corneocyte phenotypes and plasmin activity: implications for atopic dermatitis*. Br J Dermatol, 2018. **179**(2): p. 431-441.
36. Kennedy, E.A., et al., *Skin microbiome before development of atopic dermatitis: Early colonization with commensal staphylococci at 2 months is associated with a lower risk of atopic dermatitis at 1 year*. J Allergy Clin Immunol, 2017. **139**(1): p. 166-172.

37. Kezic, S., et al., *Natural moisturizing factor components in the stratum corneum as biomarkers of filaggrin genotype: evaluation of minimally invasive methods*. Br J Dermatol, 2009. **161**(5): p. 1098-104.
38. Caspers, P.J., et al., *In vivo confocal Raman microspectroscopy of the skin: noninvasive determination of molecular concentration profiles*. J Invest Dermatol, 2001. **116**(3): p. 434-42.
39. Caspers, P.J., G.W. Lucassen, and G.J. Puppels, *Combined in vivo confocal Raman spectroscopy and confocal microscopy of human skin*. Biophys J, 2003. **85**(1): p. 572-80.
40. O'Regan, G.M., et al., *Raman profiles of the stratum corneum define 3 filaggrin genotype-determined atopic dermatitis endophenotypes*. J Allergy Clin Immunol, 2010. **126**(3): p. 574-80.e1.
41. Koppes, S.A., et al., *Determination of natural moisturizing factors in the skin: Raman microspectroscopy versus HPLC*. Biomarkers, 2017. **22**(6): p. 502-507.
42. Kezic, S., et al., *Levels of filaggrin degradation products are influenced by both filaggrin genotype and atopic dermatitis severity*. Allergy, 2011. **66**(7): p. 934-40.
43. Gan, S.Q., et al., *Organization, structure, and polymorphisms of the human profilaggrin gene*. Biochemistry, 1990. **29**(40): p. 9432-40.
44. Presland, R.B., et al., *Characterization of the human epidermal profilaggrin gene. Genomic organization and identification of an S-100-like calcium binding domain at the amino terminus*. J Biol Chem, 1992. **267**(33): p. 23772-81.
45. Resing, K.A., et al., *Identification of proteolytic cleavage sites in the conversion of profilaggrin to filaggrin in mammalian epidermis*. J Biol Chem, 1989. **264**(3): p. 1837-45.
46. Hwang, J., et al., *Peptide nucleic acid (PNA) probe-based analysis to detect filaggrin mutations in atopic dermatitis patients*. Exp Dermatol, 2018. **27**(11): p. 1304-1308.
47. Smith, F.J., et al., *Loss-of-function mutations in the gene encoding filaggrin cause ichthyosis vulgaris*. Nat Genet, 2006. **38**(3): p. 337-42.
48. Fleckman, P. and A.D. Irvine, *Ichthyosis vulgaris*, in *UpToDate*, R. Corona, Editor. 2019, UpToDate.
49. Novak, N., et al., *Loss-of-function mutations in the filaggrin gene and allergic contact sensitization to nickel*. J Invest Dermatol, 2008. **128**(6): p. 1430-5.
50. Weidinger, S., et al., *Loss-of-function variations within the filaggrin gene predispose for atopic dermatitis with allergic sensitizations*. J Allergy Clin Immunol, 2006. **118**(1): p. 214-9.
51. Sergeant, A., et al., *Heterozygous null alleles in filaggrin contribute to clinical dry skin in young adults and the elderly*. J Invest Dermatol, 2009. **129**(4): p. 1042-5.
52. Baurecht, H., et al., *Toward a major risk factor for atopic eczema: meta-analysis of filaggrin polymorphism data*. J Allergy Clin Immunol, 2007. **120**(6): p. 1406-12.
53. van den Oord, R.A. and A. Sheikh, *Filaggrin gene defects and risk of developing allergic sensitisation and allergic disorders: systematic review and meta-analysis*. Bmj, 2009. **339**: p. b2433.
54. Esparza-Gordillo, J., et al., *Maternal filaggrin mutations increase the risk of atopic dermatitis in children: an effect independent of mutation inheritance*. PLoS Genet, 2015. **11**(3): p. e1005076.

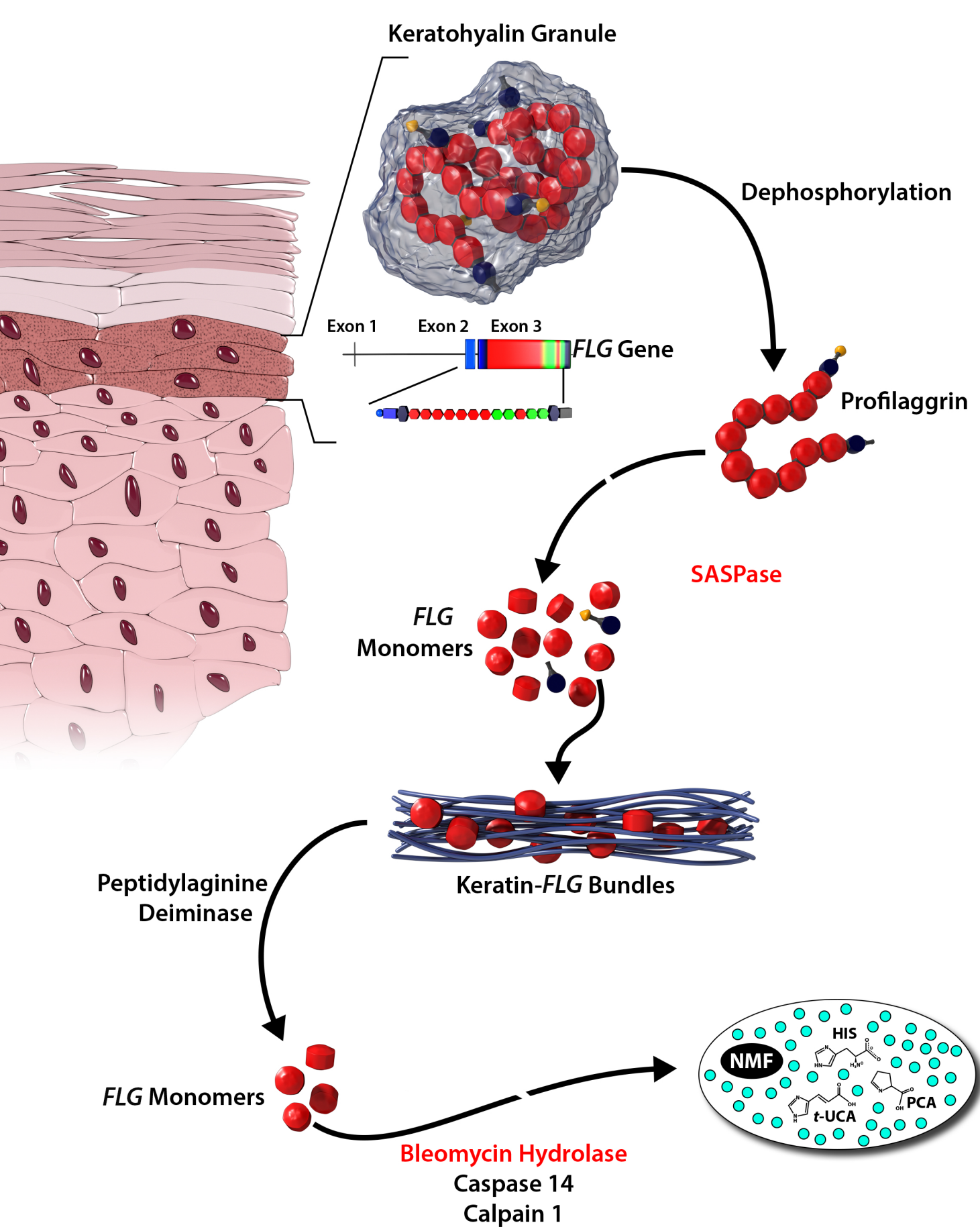
55. Czarnowicki, T., et al., *Atopic dermatitis endotypes and implications for targeted therapeutics*. J Allergy Clin Immunol, 2019. **143**(1): p. 1-11.
56. Paternoster, L., et al., *Identification of atopic dermatitis subgroups in children from 2 longitudinal birth cohorts*. J Allergy Clin Immunol, 2018. **141**(3): p. 964-971.
57. Henderson, J., et al., *The burden of disease associated with filaggrin mutations: a population-based, longitudinal birth cohort study*. J Allergy Clin Immunol, 2008. **121**(4): p. 872-7.e9.
58. Gao, P.S., et al., *Filaggrin mutations that confer risk of atopic dermatitis confer greater risk for eczema herpeticum*. J Allergy Clin Immunol, 2009. **124**(3): p. 507-13, 513.e1-7.
59. Irvine, A.D., *Fleshing out filaggrin phenotypes*. J Invest Dermatol, 2007. **127**(3): p. 504-7.
60. Fukuie, T., et al., *Palmar hyperlinearity in early childhood atopic dermatitis is associated with filaggrin mutation and sensitization to egg*. Pediatr Dermatol, 2019. **36**(2): p. 213-218.
61. Betz, R.C., et al., *Loss-of-function mutations in the filaggrin gene and alopecia areata: strong risk factor for a severe course of disease in patients comorbid for atopic disease*. J Invest Dermatol, 2007. **127**(11): p. 2539-43.
62. Heede, N.G., et al., *Anatomical patterns of dermatitis in adult filaggrin mutation carriers*. J Am Acad Dermatol, 2015. **72**(3): p. 440-8.
63. Weidinger, S., et al., *Filaggrin mutations, atopic eczema, hay fever, and asthma in children*. J Allergy Clin Immunol, 2008. **121**(5): p. 1203-1209.e1.
64. Ying, S., et al., *Lack of filaggrin expression in the human bronchial mucosa*. J Allergy Clin Immunol, 2006. **118**(6): p. 1386-8.
65. Spergel, J.M. and A.S. Paller, *Atopic dermatitis and the atopic march*. J Allergy Clin Immunol, 2003. **112**(6 Suppl): p. S118-27.
66. Paller, A.S., et al., *The atopic march and atopic multimorbidity: Many trajectories, many pathways*. J Allergy Clin Immunol, 2019. **143**(1): p. 46-55.
67. Davidson, W.F., et al., *Report from the National Institute of Allergy and Infectious Diseases workshop on "Atopic dermatitis and the atopic march: Mechanisms and interventions"*. J Allergy Clin Immunol, 2019. **143**(3): p. 894-913.
68. Tran, M.M., et al., *Predicting the atopic march: Results from the Canadian Healthy Infant Longitudinal Development Study*. J Allergy Clin Immunol, 2018. **141**(2): p. 601-607.e8.
69. Tsakok, T., et al., *Does atopic dermatitis cause food allergy? A systematic review*. J Allergy Clin Immunol, 2016. **137**(4): p. 1071-1078.
70. Asai, Y., et al., *Filaggrin gene mutation associations with peanut allergy persist despite variations in peanut allergy diagnostic criteria or asthma status*. J Allergy Clin Immunol, 2013. **132**(1): p. 239-42.
71. Brough, H.A., et al., *Peanut allergy: effect of environmental peanut exposure in children with filaggrin loss-of-function mutations*. J Allergy Clin Immunol, 2014. **134**(4): p. 867-875.e1.
72. Brough, H.A., et al., *Atopic dermatitis increases the effect of exposure to peanut antigen in dust on peanut sensitization and likely peanut allergy*. J Allergy Clin Immunol, 2015. **135**(1): p. 164-70.
73. Elhaji, Y., et al., *Filaggrin gene loss-of-function mutations constitute a factor in patients with multiple contact allergies*. Contact Dermatitis, 2019. **80**(6): p. 354-358.

74. Leung, D.Y.M., et al., *The nonlesional skin surface distinguishes atopic dermatitis with food allergy as a unique endotype*. Sci Transl Med, 2019. **11**(480).
75. Ziyab, A.H., et al., *Association of filaggrin variants with asthma and rhinitis: is eczema or allergic sensitization status an effect modifier?* Int Arch Allergy Immunol, 2014. **164**(4): p. 308-18.
76. Illi, S., et al., *The natural course of atopic dermatitis from birth to age 7 years and the association with asthma*. J Allergy Clin Immunol, 2004. **113**(5): p. 925-31.
77. Gustafsson, D., O. Sjoberg, and T. Foucard, *Development of allergies and asthma in infants and young children with atopic dermatitis--a prospective follow-up to 7 years of age*. Allergy, 2000. **55**(3): p. 240-5.
78. Sekiya, A., et al., *Compound heterozygotes for filaggrin gene mutations do not always show severe atopic dermatitis*. J Eur Acad Dermatol Venereol, 2017. **31**(1): p. 158-162.
79. Howell, M.D., et al., *Cytokine milieu of atopic dermatitis skin subverts the innate immune response to vaccinia virus*. Immunity, 2006. **24**(3): p. 341-8.
80. Fiset, P.O., D.Y. Leung, and Q. Hamid, *Immunopathology of atopic dermatitis*. J Allergy Clin Immunol, 2006. **118**(1): p. 287-90.
81. Howell, M.D., et al., *Cytokine modulation of atopic dermatitis filaggrin skin expression*. J Allergy Clin Immunol, 2009. **124**(3 Suppl 2): p. R7-r12.
82. Hata, M., et al., *Assessment of epidermal barrier function by photoacoustic spectrometry in relation to its importance in the pathogenesis of atopic dermatitis*. Lab Invest, 2002. **82**(11): p. 1451-61.
83. Jakasa, I., et al., *Increased permeability for polyethylene glycols through skin compromised by sodium lauryl sulphate*. Exp Dermatol, 2006. **15**(10): p. 801-7.
84. Jakasa, I., et al., *Altered penetration of polyethylene glycols into uninvolved skin of atopic dermatitis patients*. J Invest Dermatol, 2007. **127**(1): p. 129-34.
85. Eberlein-Konig, B., et al., *Skin surface pH, stratum corneum hydration, trans-epidermal water loss and skin roughness related to atopic eczema and skin dryness in a population of primary school children*. Acta Derm Venereol, 2000. **80**(3): p. 188-91.
86. Elias, P.M., Y. Hatano, and M.L. Williams, *Basis for the barrier abnormality in atopic dermatitis: outside-inside-outside pathogenic mechanisms*. J Allergy Clin Immunol, 2008. **121**(6): p. 1337-43.
87. Seidenari, S. and G. Giusti, *Objective assessment of the skin of children affected by atopic dermatitis: a study of pH, capacitance and TEWL in eczematous and clinically uninvolved skin*. Acta Derm Venereol, 1995. **75**(6): p. 429-33.
88. Moosbrugger-Martinz, V., et al., *Filaggrin null mutations are associated with altered circulating Tregs in atopic dermatitis*. J Cell Mol Med, 2019. **23**(2): p. 1288-1299.
89. Leitch, C.S., et al., *Filaggrin-null mutations are associated with increased maturation markers on Langerhans cells*. J Allergy Clin Immunol, 2016. **138**(2): p. 482-490.e7.
90. Bonefeld, C.M., et al., *Epidermal filaggrin deficiency mediates increased systemic T-helper 17 immune response*. Br J Dermatol, 2016. **175**(4): p. 706-12.
91. Stefanovic, N., C. Flohr, and A.D. Irvine, *The exposome in atopic dermatitis*. Allergy. **0**(0).
92. Miller, G.W. and D.P. Jones, *The nature of nurture: refining the definition of the exposome*. Toxicol Sci, 2014. **137**(1): p. 1-2.

93. Odhiambo, J.A., et al., *Global variations in prevalence of eczema symptoms in children from ISAAC Phase Three*. J Allergy Clin Immunol, 2009. **124**(6): p. 1251-8.e23.
94. Silverberg, J.I., J. Hanifin, and E.L. Simpson, *Climatic factors are associated with childhood eczema prevalence in the United States*. J Invest Dermatol, 2013. **133**(7): p. 1752-9.
95. Scott, I.R. and C.R. Harding, *Filaggrin breakdown to water binding compounds during development of the rat stratum corneum is controlled by the water activity of the environment*. Dev Biol, 1986. **115**(1): p. 84-92.
96. Katagiri, C., et al., *Changes in environmental humidity affect the water-holding property of the stratum corneum and its free amino acid content, and the expression of filaggrin in the epidermis of hairless mice*. J Dermatol Sci, 2003. **31**(1): p. 29-35.
97. Cau, L., et al., *Lowering relative humidity level increases epidermal protein deimination and drives human filaggrin breakdown*. J Dermatol Sci, 2017. **86**(2): p. 106-113.
98. Langan, S.M. and A.D. Irvine, *Childhood eczema and the importance of the physical environment*. J Invest Dermatol, 2013. **133**(7): p. 1706-9.
99. Rieko, K.-K. and N. Motonobu, *Effect of cis-urocanic acid on atopic dermatitis in NC/Nga mice*. Journal of Dermatological Science, 2016. **84**(1): p. e65-e66.
100. McAleer, M.A. and A.D. Irvine, *The multifunctional role of filaggrin in allergic skin disease*. J Allergy Clin Immunol, 2013. **131**(2): p. 280-91.
101. Lee, Y.L., et al., *Traffic-related air pollution, climate, and prevalence of eczema in Taiwanese school children*. J Invest Dermatol, 2008. **128**(10): p. 2412-20.
102. Penard-Morand, C., et al., *Long-term exposure to close-proximity air pollution and asthma and allergies in urban children*. Eur Respir J, 2010. **36**(1): p. 33-40.
103. Yi, O., et al., *Effect of environmental tobacco smoke on atopic dermatitis among children in Korea*. Environ Res, 2012. **113**: p. 40-5.
104. Kim, J., et al., *Symptoms of atopic dermatitis are influenced by outdoor air pollution*. J Allergy Clin Immunol, 2013. **132**(2): p. 495-8.e1.
105. Ahn, K., *The role of air pollutants in atopic dermatitis*. J Allergy Clin Immunol, 2014. **134**(5): p. 993-9; discussion 1000.
106. Lee, C.W., et al., *Urban particulate matter down-regulates filaggrin via COX2 expression/PGE2 production leading to skin barrier dysfunction*. Sci Rep, 2016. **6**: p. 27995.
107. Brandt, E.B. and U. Sivaprasad, *Th2 Cytokines and Atopic Dermatitis*. J Clin Cell Immunol, 2011. **2**(3).
108. Flohr, C. and J. Mann, *New insights into the epidemiology of childhood atopic dermatitis*. Allergy, 2014. **69**(1): p. 3-16.
109. Danby, S.G., et al., *The Effect of Water Hardness on Surfactant Deposition after Washing and Subsequent Skin Irritation in Atopic Dermatitis Patients and Healthy Control Subjects*. J Invest Dermatol, 2018. **138**(1): p. 68-77.
110. Torma, H., M. Lindberg, and B. Berne, *Skin barrier disruption by sodium lauryl sulfate-exposure alters the expressions of involucrin, transglutaminase 1, profilaggrin, and kallikreins during the repair phase in human skin in vivo*. J Invest Dermatol, 2008. **128**(5): p. 1212-9.

111. Engebretsen, K.A., et al., *Effect of atopic skin stressors on natural moisturizing factors and cytokines in healthy adult epidermis*. Br J Dermatol, 2018. **179**(3): p. 679-688.
112. Hachem, J.P., et al., *Serine protease activity and residual LEKTI expression determine phenotype in Netherton syndrome*. J Invest Dermatol, 2006. **126**(7): p. 1609-21.
113. Prescott, S.L., et al., *The skin microbiome: impact of modern environments on skin ecology, barrier integrity, and systemic immune programming*. World Allergy Organ J, 2017. **10**(1): p. 29.
114. Dominguez-Bello, M.G., et al., *Delivery mode shapes the acquisition and structure of the initial microbiota across multiple body habitats in newborns*. Proc Natl Acad Sci U S A, 2010. **107**(26): p. 11971-5.
115. Powers, C.E., et al., *Microbiome and pediatric atopic dermatitis*. J Dermatol, 2015. **42**(12): p. 1137-42.
116. Scharschmidt, T.C., et al., *A Wave of Regulatory T Cells into Neonatal Skin Mediates Tolerance to Commensal Microbes*. Immunity, 2015. **43**(5): p. 1011-21.
117. Scharschmidt, T.C., *Establishing Tolerance to Commensal Skin Bacteria: Timing Is Everything*. Dermatol Clin, 2017. **35**(1): p. 1-9.
118. Tsakok, T., et al., *Does early life exposure to antibiotics increase the risk of eczema? A systematic review*. Br J Dermatol, 2013. **169**(5): p. 983-91.
119. Kong, H.H., et al., *Temporal shifts in the skin microbiome associated with disease flares and treatment in children with atopic dermatitis*. Genome Res, 2012. **22**(5): p. 850-9.
120. Bieber, T., *Atopic dermatitis*. N Engl J Med, 2008. **358**(14): p. 1483-94.
121. Geoghegan, J.A., A.D. Irvine, and T.J. Foster, *Staphylococcus aureus and Atopic Dermatitis: A Complex and Evolving Relationship*. Trends Microbiol, 2018. **26**(6): p. 484-497.
122. Fleury, O.M., et al., *Clumping Factor B Promotes Adherence of Staphylococcus aureus to Corneocytes in Atopic Dermatitis*. Infect Immun, 2017. **85**(6).
123. Feuillie, C., et al., *Adhesion of Staphylococcus aureus to Corneocytes from Atopic Dermatitis Patients Is Controlled by Natural Moisturizing Factor Levels*. MBio, 2018. **9**(4).
124. Zipperer, A., et al., *Human commensals producing a novel antibiotic impair pathogen colonization*. Nature, 2016. **535**(7613): p. 511-6.
125. Iwase, T., et al., *Staphylococcus epidermidis Esp inhibits Staphylococcus aureus biofilm formation and nasal colonization*. Nature, 2010. **465**(7296): p. 346-9.
126. Clausen, M.L., et al., *Association of Disease Severity With Skin Microbiome and Filaggrin Gene Mutations in Adult Atopic Dermatitis*. JAMA Dermatol, 2018. **154**(3): p. 293-300.
127. Clausen, M.L., et al., *Staphylococcus aureus colonization in atopic eczema and its association with filaggrin gene mutations*. Br J Dermatol, 2017. **177**(5): p. 1394-1400.
128. Seite, S., et al., *Microbiome of affected and unaffected skin of patients with atopic dermatitis before and after emollient treatment*. J Drugs Dermatol, 2014. **13**(11): p. 1365-72.
129. Gonzalez, M.E., et al., *Cutaneous microbiome effects of fluticasone propionate cream and adjunctive bleach baths in childhood atopic dermatitis*. J Am Acad Dermatol, 2016. **75**(3): p. 481-493.e8.
130. Langan, S.M., C. Flohr, and H.C. Williams, *The role of furry pets in eczema: a systematic review*. Arch Dermatol, 2007. **143**(12): p. 1570-7.

131. Thorsteinsdottir, S., et al., *Domestic dog exposure at birth reduces the incidence of atopic dermatitis*. Allergy, 2016. **71**(12): p. 1736-1744.
132. Dharmage, S.C., et al., *Atopic dermatitis and the atopic march revisited*. Allergy, 2014. **69**(1): p. 17-27.
133. Flohr, C. and J. Mann, *New approaches to the prevention of childhood atopic dermatitis*. Allergy, 2014. **69**(1): p. 56-61.
134. Simpson, E.L., et al., *Emollient enhancement of the skin barrier from birth offers effective atopic dermatitis prevention*. J Allergy Clin Immunol, 2014. **134**(4): p. 818-23.
135. Chalmers, J.R., et al., *Effectiveness and cost-effectiveness of daily all-over-body application of emollient during the first year of life for preventing atopic eczema in high-risk children (The BEEP trial): protocol for a randomised controlled trial*. Trials, 2017. **18**(1): p. 343.
136. Ridd, M.J., et al., *Choice of Moisturiser for Eczema Treatment (COMET): study protocol for a randomized controlled trial*. Trials, 2015. **16**: p. 304.
137. Czarnowicki, T., et al., *Petrolatum: Barrier repair and antimicrobial responses underlying this "inert" moisturizer*. J Allergy Clin Immunol, 2016. **137**(4): p. 1091-1102.e7.
138. Stout, T.E., et al., *Recombinant filaggrin is internalized and processed to correct filaggrin deficiency*. J Invest Dermatol, 2014. **134**(2): p. 423-429.
139. Irvine, A.D., *Crossing barriers; restoring barriers? Filaggrin protein replacement takes a bow*. J Invest Dermatol, 2014. **134**(2): p. 313-314.
140. Chang, J., et al., *Association of Filaggrin Loss of Function and Thymic Stromal Lymphopoietin Variation With Treatment Use in Pediatric Atopic Dermatitis*. JAMA Dermatol, 2017. **153**(3): p. 275-281.



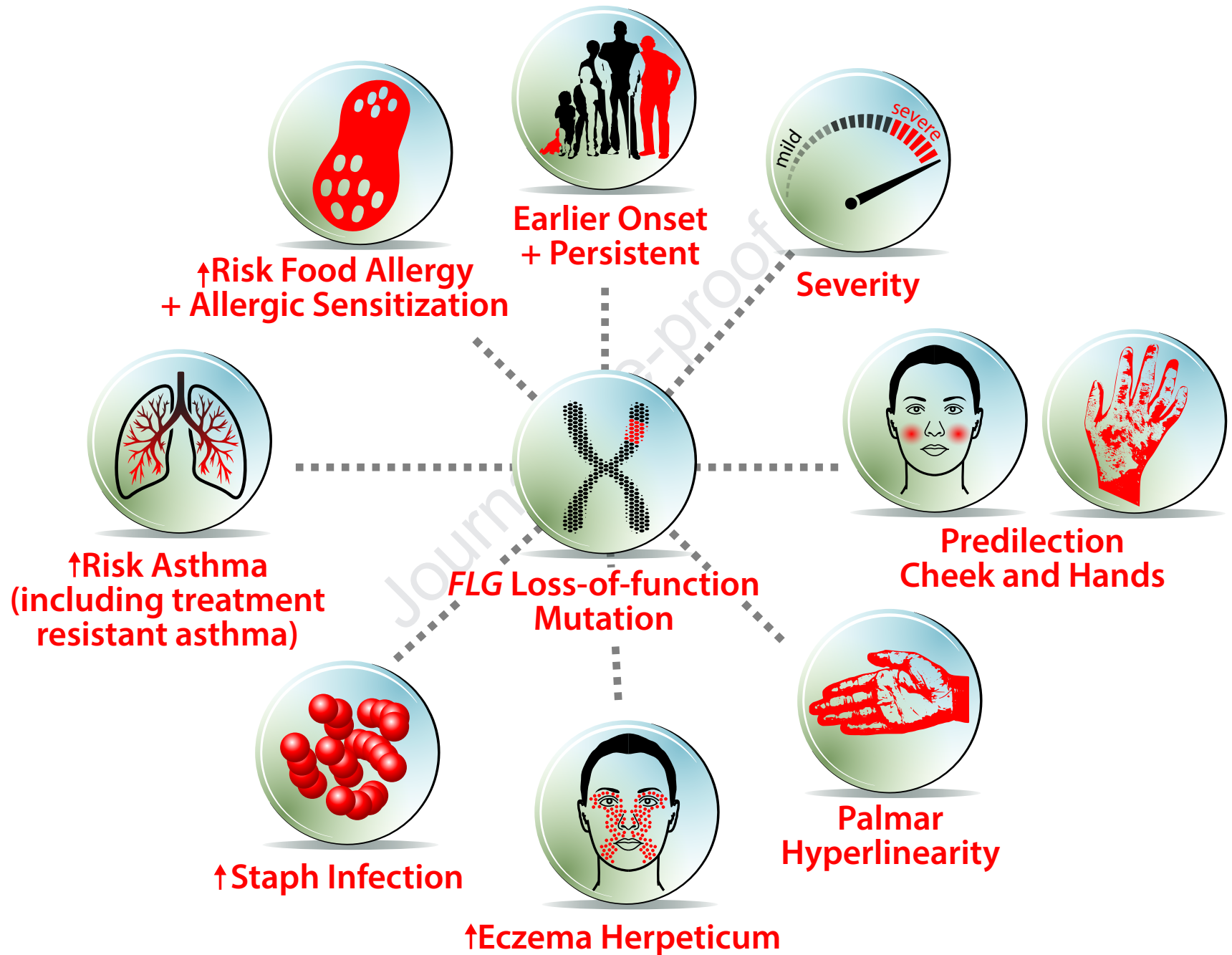


**Figure 1**

Post translational 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 dephosphorylated and cleaved by endoproteases into 10-12 filaggrin monomers which bind to keratin filaments. Filaggrin monomers are deaminated and degraded by proteases releasing a pool of hygroscopic amino acids and derivatives of the NMF.

**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.

**Table of Abbreviations**

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 Moisturising Factor  
CNV: Copy Number Variation  
RMS: Raman microspectroscopy  
HPLC: high performance liquid chromatography  
TEWL: Transepidermal water loss

## Key messages

Filaggrin is both an important risk factor for 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 downregulation of filaggrin, so that filaggrin is downregulated 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.