Filaggrin Mutations Associated with Skin and Allergic Diseases

Alan D. Irvine, M.D., W.H. Irwin McLean, Ph.D., D.Sc., and Donald Y.M. Leung, M.D., Ph.D.

Mutations in the filaggrin gene (FLG) are among the most common and profound single-gene defects identified to date in the causation and modification of disease. FLG encodes an important epidermal protein abundantly expressed in the outer layers of the epidermis. Approximately 10% of persons of European ancestry are heterozygous carriers of a loss-of-function mutation in FLG, resulting in a 50% reduction in expressed protein. The critical role of filaggrin in epidermal function underlies the pathogenic importance of this gene in common dermatologic and allergic diseases. The spectrum of such diseases encompasses monogenic disorders of keratinization through complex abnormalities of epidermal transport of lipids and allergens. FLG mutation carriers have a greatly increased risk of common complex traits, including atopic dermatitis (which affects 42% of all mutation carriers), contact allergy, asthma, hay fever, and peanut allergy. These genetic variants also influence the severity of asthma and alopecia areata and susceptibility to herpetic infection.

Filaggrin Identification and Genomics

In 1977, Beverly Dale identified a highly insoluble, histidine-rich protein that co-purified with keratin intermediate filament proteins in epidermal extracts. The purified protein condensed and aligned keratin intermediate filaments in vitro and, accordingly, was named filaggrin (for filament aggregating protein). Antibodies against this protein were used to identify both a 37-kD filaggrin protein and a high-molecular-weight proprotein, profilaggrin, now known to have a mass of more than 400 kD. Subsequent attempts to clone filaggrin messenger RNA failed to isolate the entire coding sequence, but fragments of the sequence suggested that the majority of the approximately 15-kb message encoded multiple copies of the filaggrin protein flanked by short, unique sequences. The latter include an S100-type calcium-binding domain, A and B domains of uncertain function at the N-terminal, and a unique tail sequence at the C terminal (Fig. 1).

FLG comprises three exons, the first of which is noncoding. Exon 2 encodes part of the S100 domain, and exon 3, one of the largest exons in the genome at more than 12.7 kb, encodes almost the entire profilaggrin protein (Fig. 1). The human genome reference sequence comprises 10 near-identical filaggrin repeats within exon 3, but Southern blot analysis of population samples confirmed by cloning and sequencing has shown that there are common size-variant alleles in the general population, with 10, 11, or 12 repeats. Until recently, the highly repetitive nature of exon 3 of FLG hampered routine diagnostic analysis of this gene in human disease.
*FLG* is located in the epidermal differentiation complex, a cluster of approximately 60 genes involved in epithelial differentiation, on chromosome 1q21. FLG is one of a subcluster of genes encoding seven fused S100 proteins. These proteins — filaggrin, filaggrin-2, hornerin, trichohyalin, trichohyalin-like 1, cornulin, and repetin — share a common protein-domain organization, having an S100 calcium-binding motif at the N-terminal with an extended, highly repetitive tail. The epidermal differentiation complex contains several other families of genes, including those encoding classical S100 proteins, loricrin, involucrin, small proline-rich proteins, and late cornified envelope (LCE) proteins. Copy-number variation within a cluster of LCE genes has recently been implicated as a potential causative variant underlying susceptibility to psoriasis. An unusual gain-of-function mutation in loricrin is the cause of a monogenic genodermatosis called loricrin keratoderma.

**Cellular Features of Filaggrin**

The epidermis is a complex, highly dynamic, self-renewing barrier tissue covering a very large fraction of the body. The epidermal differentiation program is illustrated in Figure 1 in the Supplementary Appendix, available with the full text of this article at NEJM.org. Cell proliferation in the epidermis is limited to epidermal stem cells in the innermost cell layer, the basal layer (Fig. 1 in the Supplementary Appendix). After cell division, daughter cells exit the cell cycle and migrate upward to form the spinous cell layers, where cell junctions are strengthened and additional keratin proteins are expressed. Closer to the skin surface, the cells of the granular layer contain dense cytoplasmic granules primarily composed of profilaggrin, with other protein components required for the formation of squames, the flattened, dead cells of the outermost stratum corneum that are responsible for the barrier function of the skin (Fig. 1 in the Supplementary Appendix). Two important barrier functions of this stratified, cornified squamous epithelium are to prevent water loss through the huge surface area of the human body and to block the entry of foreign substances (pathogens, antigens, allergens, and chemical irritants) from the external environment.

*Profilaggrin* is expressed late in the epidermal differentiation program, in the granular cell layer.
ers of stratified, cornified epithelia (Fig. 1 in the Supplementary Appendix). The cytoplasm of the epidermal granular cells is packed with densely staining keratohyalin granules, the main constituent of which is profilaggrin — a fact confirmed by the complete absence of granules in persons carrying homozygous null mutations in FLG (Fig. 1 in the Supplementary Appendix).6 These granules act as a reservoir of inactive profilaggrin, along with other proteins that are important for squame formation and maturation, such as loricrin. The transitional zone is essentially the uppermost layer of viable granular cells that are in the final process of differentiation into the flattened, tightly packed, and chemically cross-linked anuclear keratinocytes (squames), which make up the stratum corneum. A “bricks and mortar” model of the stratum corneum has been proposed,15 in which the flattened residual cells (squames) act as the bricks and the cornified cell envelope acts as the mortar. Together, they form a matrix of highly ordered and specialized lipids.16 The end products of filaggrin processing are found within the residual cytoplasm of these squames.

In the transitional cells, profilaggrin processing is initiated. The precise triggering event is unclear, but it is likely to involve dephosphorylation of the proprotein in concert with derepression of a protease cascade that leads to rapid liberation of the functionally active filaggrin monomers.3 A number of proteases have been shown to be involved in this process, with definitive data coming from studies of knockout and transgenic mice.17–20 Very recently, skin-specific retroviral-like aspartic protease has been shown to be specifically expressed in the transitional layer and to be the key enzyme responsible for cleavage of the individual filaggrin repeat units.23 Absence of the serine protease inhibitor LEKTI, encoded by SPINK5, leads to premature processing of profilaggrin and a complex phenotype that includes ichthyosis, hair-shaft abnormalities, and atopy (the Netherton syndrome).22

In the squames, filaggrin undergoes further modification. Fragments of filaggrin have been identified in covalent cross-links mediated by transglutaminase23; therefore, the protein contributes to the formation of the cornified cell envelope — the highly impermeable, proteolipid mortar structure of the stratum corneum. Arginine residues in filaggrin are converted to citrulline24; this is thought to facilitate further proteolysis into short peptides and, ultimately, a pool of hygroscopic amino acids and derivatives thereof, known as natural moisturizing factor (NMF).25 This process involves caspase 14 and other proteases.26,27 Important derivatives of amino acids include trans-urocanic acid and pyrrolidone carboxylic acid. Urocanic acid provides protection against ultraviolet radiation28 and modulates immune function.29 Pyrrolidone carboxylic acid is a chemical derivative of glutamine.30 Profilaggrin is one of the most histidine-rich and glutamine-rich proteins in the human genome, and it acts as a pool of these amino acids, which in turn modulate the pH of the stratum corneum, exert intracytoplasmic humectant activity (i.e., promote the retention of moisture), and possibly exert antimicrobial activity against staphylococcus.31 Measurement of epidermal NMF by means of in vivo confocal Raman spectroscopy has been shown to strongly correlate with filaggrin null mutation genotype.32 Measurements of urocanic acid and pyrrolidone carboxylic acid in epidermal tape strips strongly correlate with filaggrin genotype.33

The multifunctionality of filaggrin is clearly illustrated by comparing flaky tail mice (which have a loss-of-expression mutation in FLG) with caspase 14–knockout mice. In flaky tail mice, loss of profilaggrin and all downstream events leads to aberrant biogenesis of the stratum corneum (ichthyosis) as well as abnormally dry skin (xerosis).34 In contrast, caspase 14–null skin, in which profilaggrin-to-filaggrin processing is normal but filaggrin-to-NMF processing is absent, have xerosis and sensitivity to ultraviolet light without ichthyosis.26!

Filaggrin deficiency has been experimentally shown to lead to a failure in the barrier function of the skin. The epidermis of filaggrin-deficient mice allows passive transfer of protein allergens. In cultures of human organotypic keratinocytes, the knockdown of FLG expression by RNA interference facilitates the uptake of fluorescent dyes and heavy-metal tracers.35,36 It is unclear how filaggrin deficiency leads to this defect in barrier function in terms of the bricks-and-mortar model; however, because assembly of lipid and protein components of the stratum corneum must be coordinated, bidirectional signaling should exist between these systems. Knockout of 12-lipoxygenase, an enzyme essential for lipid biogenesis in the stratum corneum,37 or of ATP-binding cassette transporter A12 (ABCA12), a lipid transporter,38
leads to aberrant profilaggrin processing, suggestive of lipid-protein signaling. It has been shown that maintaining a low pH in the stratum corneum is essential for lipid secretion and assembly.\(^3^9\)

<table>
<thead>
<tr>
<th>MONOGENIC DISEASE</th>
<th>DISEASES ASSOCIATED WITH FILAGGRIN MUTATIONS</th>
</tr>
</thead>
</table>
| Ichthyosis vulgaris (Fig. 2) is one of the most common mendelian (single-gene) diseases, and it is the most prevalent disorder of keratinization, with a population prevalence of at least 1 case in 250 persons.\(^4^0\) It is characterized by the postnatal appearance of dry, flaking skin, the severity of which varies seasonally; roughening of skin around the hair follicles (keratosis pilaris), particularly on the upper outer arms, thighs, and outer cheeks; and increased skin markings (termed hyperlinearity) on the palms and soles. Ichthyosis vulgaris, which has a well-recognized association with atopic dermatitis,\(^4^1,4^2\) is inherited in an autosomal semi-dominant pattern; that is, carriers of a single mutation have no disease or mild disease, whereas carriers of two mutations have more marked disease manifestations.\(^6\) The discovery that loss-of-function mutations in FLG underlie ichthyosis vulgaris clarified the inheritance pattern and opened the way to further explanation of the role of FLG in complex diseases.\(^6\)

Since the identification of the 2 original mutations (R501X and 2282del4), an additional 47 mutations have been reported, all of which predict loss of function with no translated protein (Fig. 3).\(^4^2\) In contrast to the vast majority of variants linked to complex traits, which are very often single-nucleotide polymorphisms with no clear function, all filaggrin variants are nonsense or frameshift mutations in a protein-encoding exon and are therefore readily identified as causative variants without functional analysis.\(^9\) The complex genetic architecture of the FLG locus has become apparent, and it includes a combination of recurrent and family-specific mutations. The recurrent mutations are also specific to certain ethnic groups, with distinct profiles seen in the European and Asian populations that have been well studied thus far (Fig. 3).\(^4^3\)

In addition to having a causal role in ichthyosis vulgaris, FLG mutations appear to have a modifying effect on the single-gene disorder X-linked recessive ichthyosis. In small studies, persons who carry a mutation in FLG in addition to a mutation in the STS gene appear to have a more severe phenotype than do their affected siblings who have wild-type FLG.\(^4^4\) A similar modifier effect may also be relevant for the keratinization disorder pachyonychia congenita.\(^4^5\) Given the high population frequency of FLG null alleles and the importance of the filaggrin protein in the stratum corneum, it is likely that FLG mutations will prove to be modifiers of other disorders of keratinization.

**Atopic Dermatitis**

Atopic dermatitis is an itchy inflammatory skin disease that affects approximately 11% of children in the United States\(^4^6\) and up to 25% in the United Kingdom,\(^4^7,4^8\) making it the most common chronic inflammatory skin disease of early childhood. Atopic dermatitis can cause considerable illness,\(^4^9\) with substantial associated family stress and social and financial burden.\(^5^0\) A systematic review estimated the annual direct and indirect costs of atopic dermatitis in the United States at $364 million to $3.8 billion.\(^5^1\) The disorder is strongly associated with food allergies, asthma, and allergic rhinitis in later life (i.e., the so-called atopic march).\(^5^2\)

FLG mutations were strongly associated with atopic dermatitis in an Irish population and with atopic dermatitis plus asthma in a Scottish population.\(^5^3\) These findings have been widely replicated.\(^5^4-5^8\) Meta-analyses of studies involving many thousands of patients have confirmed these associations,\(^5^6,5^9\) with an overall odds ratio for atopic dermatitis ranging from 3.12 to 4.78.\(^5^7,5^9\) Stronger associations have been observed for dermatologist-diagnosed and moderate-to-severe atopic dermatitis, suggesting that patients with atopic dermatitis who carry FLG mutations may have a predisposition for a distinct profile of disease as compared with patients without such mutations. This interpretation is supported by additional studies showing that patients with atopic dermatitis who carry FLG mutations have more persistent disease,\(^6^0\) a higher incidence of skin infections with herpes virus (eczema herpeticum),\(^6^1\) and a greater risk of multiple allergies\(^5^7,5^9,6^0\) and asthma\(^6^0,6^2\) than patients with atopic dermatitis without such mutations. Further work has shown that in addition to having a primary effect on atopic dermatitis, FLG mutations are associated with both irritant contact dermatitis\(^6^3\) and nickel allergy\(^6^4\) (Table 1 in the Supplementary Appendix). In alopecia areata, FLG mutations appear to have a modifier effect associated with more severe outcomes.\(^6^5\)
Asthma, Allergic Rhinitis, and Food Allergy

In addition to their role in atopic dermatitis, filaggrin loss-of-function mutations confer genetic risk for several other complex diseases (Fig. 4, and Table 1 in the Supplementary Appendix). The association of FLG mutations with asthma is complex. In large population studies, FLG loss-of-function mutations have conferred an overall asthma risk ranging from 1.48 to 1.79, but this effect was limited to subjects with atopic dermatitis or a history of it. Filaggrin is not expressed in the respiratory epithelia. The assumption is therefore that atopic dermatitis is a causal risk factor for asthma and systemic allergen sensitization in the context of FLG mutations. The mechanisms of this relationship are as yet unclear. Increased allergen and pathogen penetration through the stratum corneum, followed by stimulation of keratinocyte-derived thymic stromal lymphopoietin, an interleukin-7–like cytokine, in inflamed epidermis may lead to distal effects in the lung.

The relationship between atopic dermatitis, asthma, and FLG mutations is complex; it is now clear that among atopic dermatitis patients, those with FLG mutations have a much greater risk of asthma than do those without FLG mutations, whereas among asthma patients, those with FLG mutations have a more difficult course and more frequent exacerbations (Fig. 4). The complex and possibly distinct phenotype of “asthma plus atopic dermatitis” thus requires further examination. A strong association with allergic rhinitis has also been noted in some population studies.

Peanut allergy is a strongly heritable condition, with a monozygotic concordance of 64% as compared with 7% in dizygotic twins. Our recent studies show that FLG mutations confer an overall odds ratio of 5.3 for peanut allergy (defined by a positive food challenge), with a residual odds ratio of 3.8 when corrected for atopic dermatitis.
These data suggest a barrier defect that facilitates enhanced exposure of peanut allergen to antigen-presenting cells, even in the absence of atopic dermatitis.

**Disease Mechanisms of Filaggrin Mutations**

The mechanisms by which these genetic defects lead to human disease have been intensely investigated. Ichthyosis vulgaris, the exemplar mendelian disease of filaggrin deficiency, is characterized by dry, flaking skin (Fig. 2). FLG mutations are the major determinants of the levels of the hygroscopic and humectant filaggrin breakdown products, pyrrolidone carboxylic acid and urocanic acid, and other components of NMF.32,33 Thus, the mechanism through which filaggrin deficiency causes abnormally dry skin in ichthyosis vulgaris is clear (Fig. 5). The mechanisms through which filaggrin deficiency may lead to the disordered epithelial differentiation manifested clinically as dry and flaky skin are discussed below.

Atopic dermatitis, the primary complex disease associated with filaggrin deficiency, is characterized by dry skin, a cutaneous barrier defect, enhanced allergen priming, susceptibility to cutaneous bacterial colonization and infection (especially *Staphylococcus aureus* infection), and cutaneous inflammation driven by type 2 helper T (Th2) cells.24 Although atopic dermatitis was, for many years, considered to be a primarily immunologically driven disease with a secondary barrier defect (the so-called inside-outside hypothesis), some investigators had hypothesized that the primary defect was in the skin barrier (the outside-inside hypothesis).75,76 Abnormal barrier function of the skin has long been noted in ichthyosis vulgaris, even in the absence of atopic dermatitis.77,78 The remarkable association of FLG mutations with atopic dermatitis has validated the outside-inside hypothesis.

FLG mutations may play a role in the development of each of the key features of atopic dermatitis (Fig. 5). Although it is widely held that these mutations lead to a functional barrier defect,79 the mechanisms of this defect are still being elucidated. Important insights have been gained from complementary studies of human subjects and murine models. These studies have shown that filaggrin deficiency may contribute to disease pathogenesis through a variety of differentiation-specific effects (Fig. 5). Although studies of mice have limitations in their relevance to complex human disease (because of the potential influence of strain, immunologic background, epidermal differentiation complex haplotype, and the fact that murine skin is much hairier than human skin), useful insights have been gained from murine studies. All studies of the flaky tail mouse, which carries a directly analogous loss-of-function mutation in *flg*, have shown abnormalities in barrier function, with lowered irritability thresholds and enhanced cutaneous allergen penetration.80-82 This minor baseline abnormality is greatly enhanced by exposure to allergens and to standard irritants such as sodium lauryl sulfate.80

The integrity of the stratum corneum barrier is primarily conferred by extracellular lipid lamellae. Filaggrin deficiency may contribute to defective lipid lamellae through a number of mechanisms. In the transitional zone of the stratum corneum, filaggrin deficiency impairs filament aggregation, which in turn impairs the matura-
A diagram showing various genetic variations and their positions on a chromosome. The genetic variations include deletions, insertions, and single nucleotide polymorphisms (SNPs). The diagram is divided into European and Asian variants. Each variant is connected to a specific position on the chromosome, indicating its location.

B shows a close-up of the region with a focus on specific genetic variations such as R501X and R4306X.

C presents a pie chart comparison between Atopic dermatitis in Singapore and Atopic dermatitis in Ireland. The pie chart indicates the frequency of different genetic variations associated with these conditions, with 2282del4 being the most common variant in both regions.
tion and excretion of extracellular lamellar bodies.\textsuperscript{36} Tight junctions are critical in sealing epidermal cell-to-cell integrity\textsuperscript{83}; these junctions appear to be reduced in number in filaggrin-deficient persons.\textsuperscript{36} Similarly, such persons have a decreased density of corneodesmosin, the major protein component of corneodesmosomes (organelles that are critical for stratum corneum cell-to-cell adhesion).\textsuperscript{36}

The acidifying effect of filaggrin breakdown products\textsuperscript{84} is probably important. The elevation in skin-surface pH that is observed in FLG-deficient

\textbf{Figure 4. Filaggrin Haploinsufficiency and Increased Risk of Several Complex Traits.}

Filaggrin haploinsufficiency is defined as a 50\% reduction in the expression of the filaggrin protein. The odds ratios are for the risk of peanut allergy, asthma, or atopic dermatitis as compared with the risk in the absence of filaggrin mutation. The odds ratios listed for atopic dermatitis and asthma are from meta-analyses involving several thousand patients. FLG mutations confer an overall risk of asthma of 1.5, but this risk is restricted to patients with atopic dermatitis. The odds ratio for the complex phenotype of asthma plus atopic dermatitis is 3.3. The odds ratio for peanut allergy is based on the only available data, from a single study.
persons suggests an alternative mechanism for the barrier defect. Neutral or alkaline pH is optimal for several serine proteases. Activation of kallikrein serine proteases has major downstream consequences, including activation of the plasminogen activator type 2 receptor-mediated blockade of lamellar-body secretion. Activation of serine proteases may also directly drive kallikrein S-mediated Th2 inflammation, even in the absence of allergen priming.

An elevation in the pH of the stratum corneum may lead to enhanced S. aureus adhesion and proliferation through pH-dependent and possibly pH-independent mechanisms. Elevated skin-surface pH increases the activity of several proteases that cleave proforms of interleukin-1, possibly contributing to epidermal inflammation and further barrier destruction.
multiplication. In addition, urocanic acid and pyrrolidone carboxylic acid may contribute a specific antistaphylococcal effect by directly inhibiting bacterial expression of iron-regulated surface determinant protein A, which promotes bacterial adhesion to squames.

Although FLG mutations lead to the primary abnormalities as outlined above, it is important to note that atopic dermatitis develops in only approximately 42% of all FLG heterozygotes; thus, both genetic modifiers and environmental factors must be important. Epidemiologic studies suggest that early exposure to cats may be an important environmental factor. In addition, the presence of elder siblings increases the risk of atopic dermatitis in FLG mutation carriers. Thus, different causal pathways between genes and the environment may be important in patients with atopic dermatitis who carry such mutations as opposed to those who do not. Epistatic genetic effects, including effects of the plausible candidate genes SPINK5 and KLK7, have not yet been identified.

### Immunomodulation of Filaggrin

Although FLG mutations are strong genetic determinants of atopic dermatitis, the majority of children with atopic dermatitis who carry FLG mutations outgrow the skin disease after 12 years of age, suggesting that there may be immunologic determinants of low FLG expression. The barrier function of the skin appears to be reduced in all patients with atopic dermatitis, and decreased filaggrin expression is commonly found even in those who do not carry FLG mutations. Consistent with these observations is the finding that the Th2 cytokines interleukin-4, interleukin-13, and interleukin-25, which are involved in allergic responses, reduce FLG expression by keratinocytes in vitro. The in vivo significance of these findings is supported by the observation that mice with overexpression of STAT6, a transcription factor that mediates the action of interleukin-4 and interleukin-13, have reduced expression of filaggrin. Interleukin-4 and interleukin-13 may inhibit filaggrin expression through down-regulation of keratinocyte differentiation by modulating the calcium-sensing protein S100A11. Increased protease activity in the skin's inflammatory response can lead to excessive degradation of filaggrin by affecting the processing of profilaggrin. Diverse immune and inflammatory responses probably modulate filaggrin expression and contribute to skin-barrier dysfunction.

---

**Therapeutic Considerations and Personalized Medicine**

The discovery of the prominent role of inherited filaggrin deficiency in ichthyosis vulgaris and atopic disease has brought the barrier function of the skin to the center stage in efforts to develop treatments for these common conditions. The first approach is immunomodulation. Topical anti-inflammatory agents have been shown to reverse the reduced filaggrin expression found in lesional atopic dermatitis, a finding that is consistent with the concept that inflammation can reduce filaggrin expression. Given that Th2 cytokines can inhibit filaggrin expression, interventions that interfere with allergic immune responses may also enhance filaggrin expression in the skin of patients with atopic dermatitis and thereby ameliorate the inherent barrier defect.

Second, high-throughput screening is being used to identify compounds that up-regulate filaggrin expression, by targeting regulatory pathways that control the expression of filaggrin and other skin-barrier proteins. However, FLG is a late-differentiation–specific gene that is not normally expressed in monolayer keratinocyte cultures, making it difficult to develop a high-throughput screening assay.

A third strategy is to target the protein-translation machinery in order to “ignoring” nonsense mutations and thereby restore protein expression. This is particularly applicable to persons who are homozygotes or compound heterozygotes for FLG, who have the most severe cases of ichthyosis vulgaris or atopic dermatitis (about 1% of the general population). This approach, already in development for cystic fibrosis and Duchenne’s muscular dystrophy, has the potential to treat a subset of patients with many other genetic disorders and therefore has wider applicability.

Pharmacologic interventions that directly target filaggrin are a long way from clinical application, but in the future, one might envision using inexpensive, rapid genetic testing for filaggrin variants, coupled with an appropriate treatment regimen designed to enhance the barrier func-
tion of the skin, as an early intervention or preventive measure in persons at risk for atopic dermatitis. It remains to be seen whether restoration or augmentation of filaggrin expression in patients with preexisting atopic dermatitis will be therapeutic. Nevertheless, the filaggrin discovery has given rise to the new field of skin-barrier enhancement therapy, which represents an exciting opportunity to tackle common diseases.

Disclosure forms provided by the authors are available with the full text of this article at NEJM.org.

We thank the patients and families who have contributed so importantly to our studies, Boyd Jacobson for superb assistance with earlier versions of the figures, and Maureen Sandoval for assistance in preparation of an earlier draft of this manuscript.


