REVIEW ARTICLE

MECHANISMS OF DISEASE

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.[1] 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.[2] 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.

From the National Children's Research Centre and Department of Paediatric Dermatology, Our Lady's Children's Hospital, and the Department of Clinical Medicine, Trinity College — both in Dublin (A.D.I.); the Division of Molecular Medicine, Colleges of Life Sciences and Medicine, Dentistry, and Nursing, University of Dundee, Dundee, United Kingdom (W.H.I.M.); the Department of Pediatrics, National Jewish Health, Denver (D.Y.M.L.); and the Department of Pediatrics, University of Colorado Denver, Aurora (D.Y.M.L.). Address reprint requests to Dr. Leung at National Jewish Health, 1400 Jackson St. (K926i), Denver, CO 80206, or at leungd@njhealth.org.

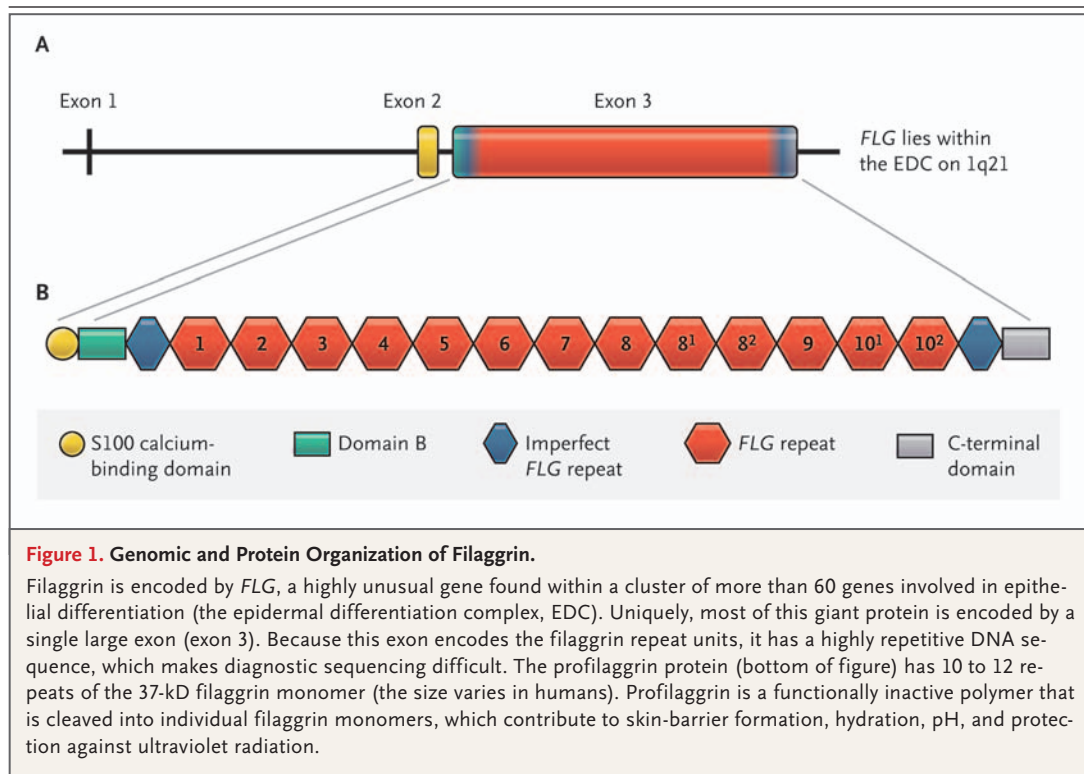Drs. Irvine and McLean contributed equally to this article.

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.[3] The purified protein condensed and aligned keratin intermediate filaments in vitro and, accordingly, was named filaggrin (for filament aggregating protein).[4] Antibodies against this protein were used to identify both a 37-kD filaggrin protein and a high-molecular-weight proprotein, profilaggrin,[5] now known to have a mass of more than 400 kD.[6] 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.[7] 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.[1] 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).[1] The human genome reference sequence comprises 10 near-identical filaggrin repeats within exon 3, but Southern blot analysis of population samples,[8] confirmed by cloning and sequencing,[9] 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.[6,9]

Figure 1. Genomic and Protein Organization of Filaggrin.

Filaggrin is encoded by *FLG*, a highly unusual gene found within a cluster of more than 60 genes involved in epithelial differentiation (the epidermal differentiation complex, EDC). Uniquely, most of this giant protein is encoded by a single large exon (exon 3). Because this exon encodes the filaggrin repeat units, it has a highly repetitive DNA sequence, which makes diagnostic sequencing difficult. The profilaggrin protein (bottom of figure) has 10 to 12 repeats of the 37-kD filaggrin monomer (the size varies in humans). Profilaggrin is a functionally inactive polymer that is cleaved into individual filaggrin monomers, which contribute to skin-barrier formation, hydration, pH, and protection against ultraviolet radiation.

FLG is located in the epidermal differentiation complex, a cluster of approximately 60 genes involved in epithelial differentiation, on chromosome 1q21.[10] *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.[1] 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.[11] An unusual gain-of-function mutation in loricrin is the cause of a monogenic genodermatosis called loricrin keratoderma.[12]

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.[1,13]

Profilaggrin is expressed late in the epidermal differentiation program, in the granular cell lay-

ers of stratified, cornified epithelia[14] (Fig. 1 in the Supplementary Appendix). The cytoplasm of the epidermal granular cells is packed with dense-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.[1] 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.[21] 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 transglutaminase[23]; 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 citrulline[24]; 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 radiation[28] 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 animals, 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.[39]

DISEASES ASSOCIATED WITH FILAGGRIN MUTATIONS

MONOGENIC DISEASE

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.[40] 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,[41,42] 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).[42] 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).[43]

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*.[44] A similar modifier effect may also be relevant for the keratinization disorder pachyonychia congenita.[45] 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[46] and up to 25% in the United Kingdom,[47,48] making it the most common chronic inflammatory skin disease of early childhood. Atopic dermatitis can cause considerable illness,[49] with substantial associated family stress and social and financial burden.[50] A systematic review estimated the annual direct and indirect costs of atopic dermatitis in the United States at $364 million to $3.8 billion.[51] The disorder is strongly associated with food allergies, asthma, and allergic rhinitis in later life (i.e., the so-called atopic march).[52]

FLG mutations were strongly associated with atopic dermatitis in an Irish population and with atopic dermatitis plus asthma in a Scottish population.[53] These findings have been widely replicated.[54-58] Meta-analyses of studies involving many thousands of patients have confirmed these associations,[56,59] with an overall odds ratio for atopic dermatitis ranging from 3.12 to 4.78.[57,59] 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,[60] a higher incidence of skin infections with herpes virus (eczema herpeticum),[61] and a greater risk of multiple allergies[57,59,60] and asthma[60,62] 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[63] and nickel allergy[64] (Table 1 in the Supplementary Appendix). In alopecia areata, *FLG* mutations appear to have a modifier effect associated with more severe outcomes.[65]

Figure 2. Clinical and Immunohistochemical Features of Ichthyosis Vulgaris.

Ichthyosis vulgaris is a semidominant mendelian disease; affected persons with one loss-of-function filaggrin mutation have mild disease or are clinically unaffected, but those with two loss-of-function mutations have marked disease. The disease is characterized clinically by mild, superficial scaling that is best seen with tangential light (Panel A). An unusually severe case of the condition is shown in Panel B. Affected persons who carry two loss-of-function filaggrin mutations have marked palmar hyperlinearity, best seen over the thenar eminence (Panel C). In this case, the patient was a coal miner, and his exposure to coal dust accentuated the hyperlinearity. Immunohistochemical staining for filaggrin shows that as compared with unaffected persons (Panel D), patients with two loss-of-function mutations have no staining in the granular layer and stratum corneum (Panel E). In addition, filaggrin deficiency results in larger keratinocytes. Photograph in Panel C courtesy of Prof. Peter Hull.

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,[60,66] but this effect was limited to subjects with atopic dermatitis or a history of it. Filaggrin is not expressed in the respiratory epithelia.[67] The assumption is therefore that atopic dermatitis is a causal risk factor for asthma and systemic allergen sensitization in the context of *FLG* mutations.[68] 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.[69]

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,[52,60,66] whereas among asthma patients, those with *FLG* mutations have a more difficult course and more frequent exacerbations (Fig. 4).[70] The complex and possibly distinct phenotype of "asthma plus atopic dermatitis" thus requires further examination.[71] A strong association with allergic rhinitis has also been noted in some population studies.[59,66]
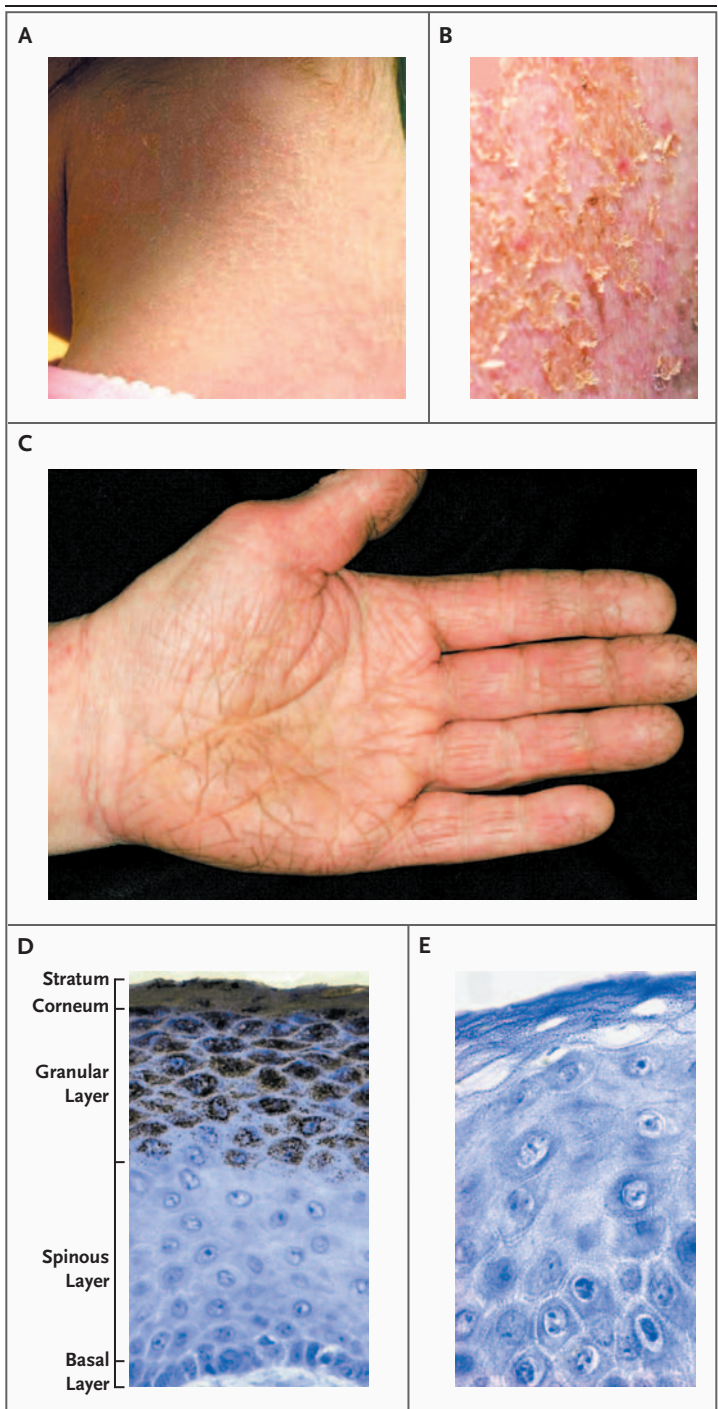
Peanut allergy is a strongly heritable condition, with a monozygotic concordance of 64% as compared with 7% in dizygotic twins.[72] Our recent studies show that *FLG* mutations confer an overall odds ratio of 5.3 for peanut allergy (defined by a positive food challenge), with a residual odds ratio of 3.8 when corrected for atopic dermatitis.[73]

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.[74] 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

Figure 3 (facing page). Variation in Filaggrin Mutations among Ethnic Groups and Other Populations.

Including the 2 initially reported mutations (R501X and 2282del4), 49 truncating mutations throughout the length of the profilaggrin molecule have been described, with many European-specific and Asian-specific mutations (Panel A). Mutations that are recurrent in these populations are indicated in red, and rare or family-specific mutations are in black. All mutations are either nonsense mutations or out-of-frame insertions or deletions that are predicted to cause loss of function. Regardless of site, these mutations result in the absence of processed filaggrin in the stratum corneum because of the instability of truncated profilaggrin. At least 3 variants in filaggrin copy number are recognized, with 10, 11, or 12 repeat alleles present in normal populations (Panel B). These copy-number variants may also influence disease. The common R501X mutation occurs on an 11-repeat allele; 2282del4 occurs on the 10-repeat allele (Panel B). Some mutations (R4306X and E4265X) occur on a second copy of repeat 10 that is specific to the 12-repeat allele (Panel B). The architecture of *FLG* risk alleles varies considerably among populations (Panel C). For example, among patients in Ireland who have atopic dermatitis, 2 recurrent mutations (R501X and 2282del4) account for 80% of the total risk alleles, and 3 other mutations account for an additional 16%. In contrast, the spectrum of risk alleles among such patients in Singapore is much wider, with 16 recurrent mutations contributing from 1 to 24% of total risk.

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-
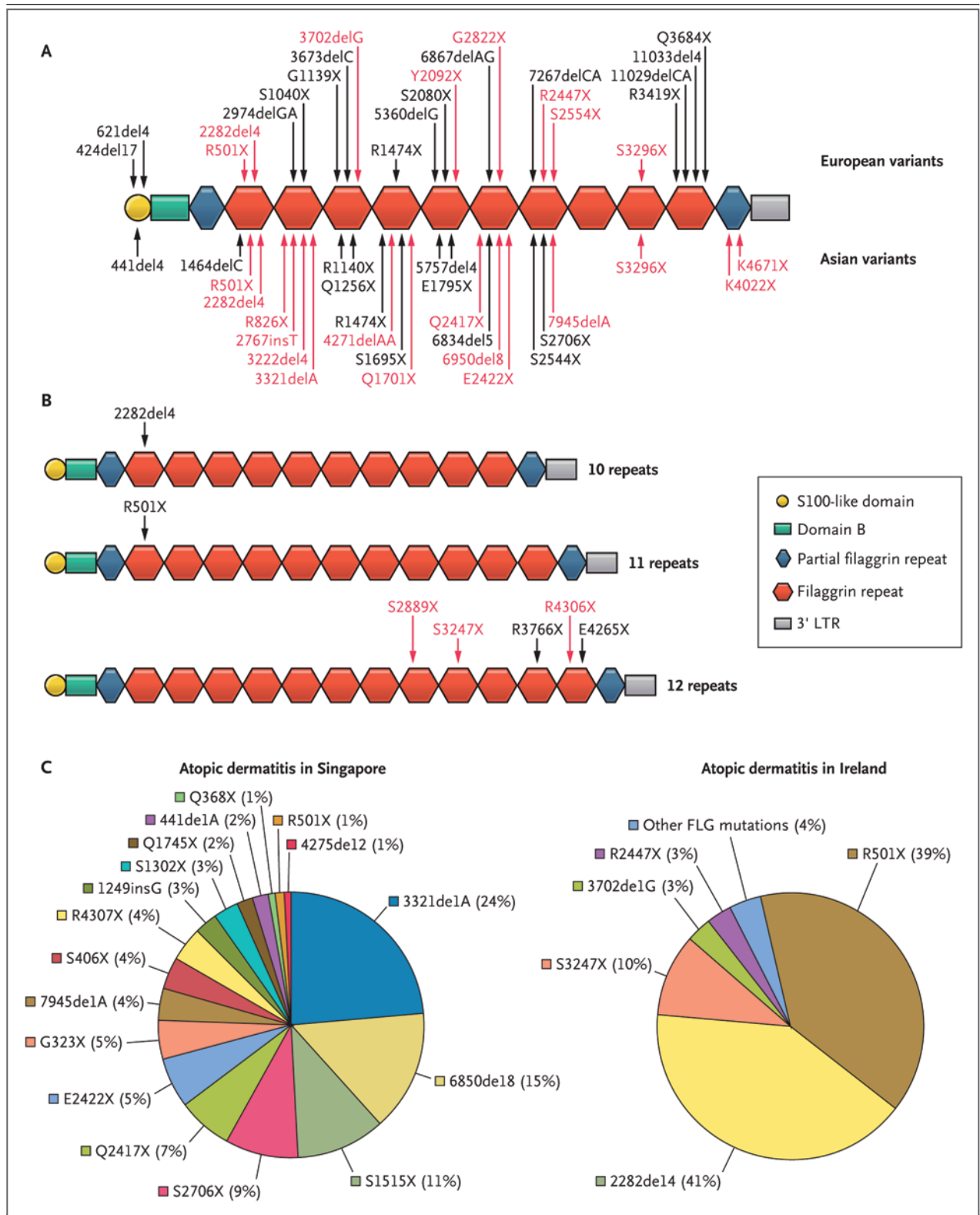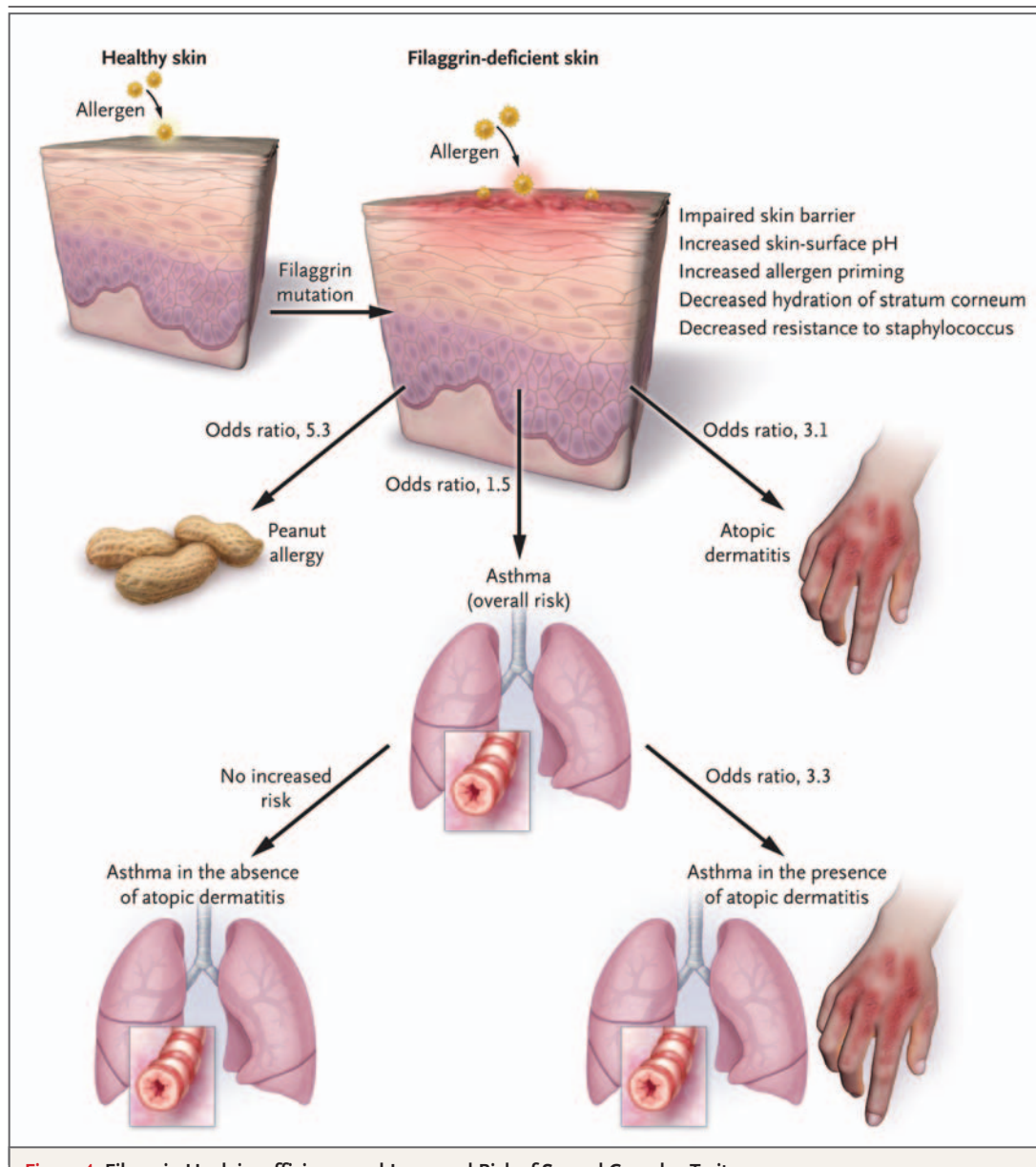
Figure 4. Filaggrin Haploinsufficiency and Increased Risk of Several Complex Traits.
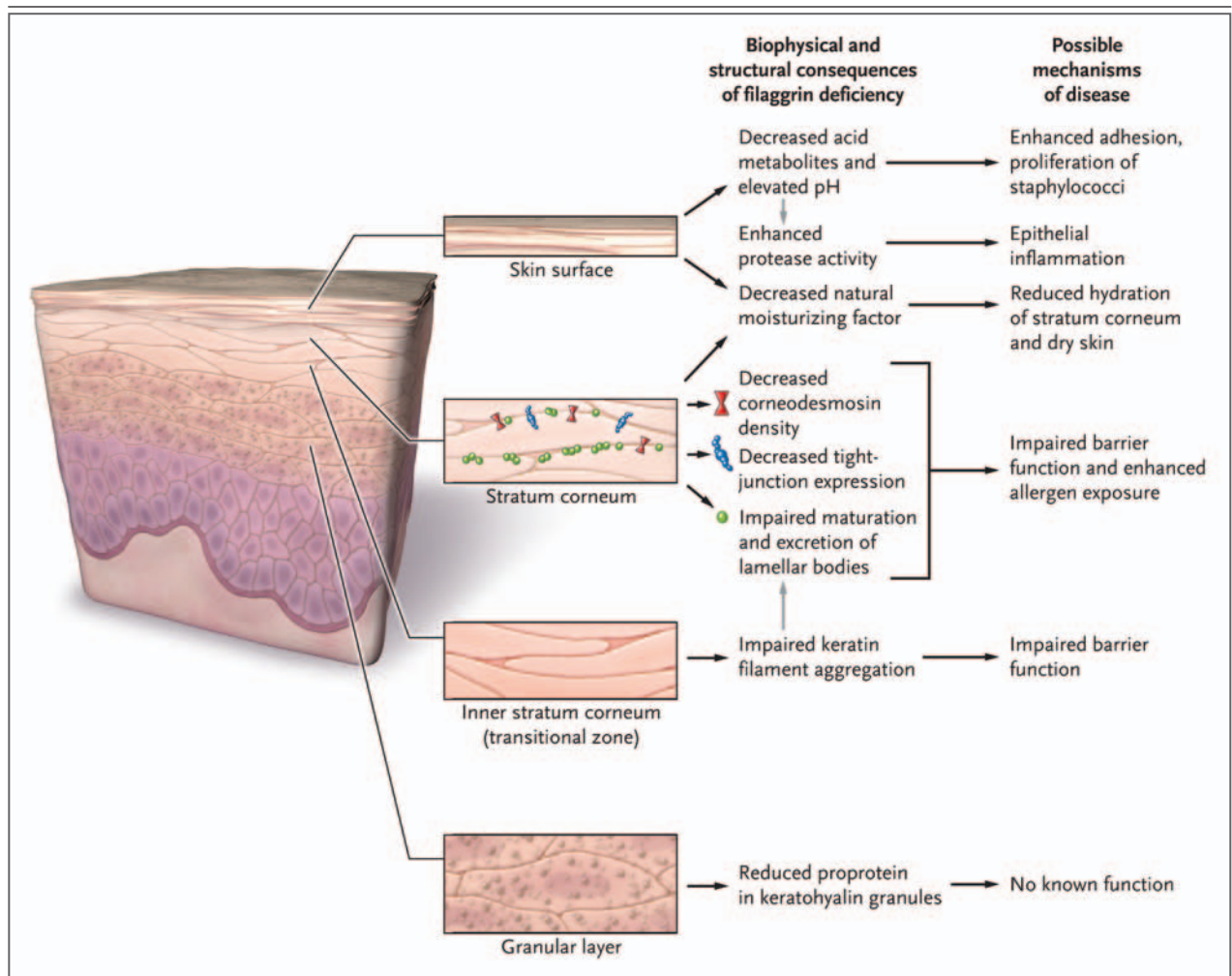
Filaggrin haploinsufficiency is defined as a 50% reduction in the expression of the filaggrin protein. The odds ratios are for the risk of peanut allergy, asthma, or atopic dermatitis as compared with the risk in the absence of filaggrin mutation. The odds ratios listed for atopic dermatitis and asthma 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.

tion and excretion of extracellular lamellar bodies.[36] Tight junctions are critical in sealing epidermal cell-to-cell integrity[83]; these junctions appear to be reduced in number in filaggrin-deficient persons.[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).[36]

The acidifying effect of filaggrin breakdown products[84] is probably important. The elevation in skin-surface pH that is observed in *FLG*-deficient

Figure 5. Filaggrin Deficiency and Possible Mechanisms of Disease.

Filaggrin haploinsufficiency results in a number of differentiation-specific structural, biophysical, and functional changes within the stratum corneum that are likely to be directly related to disease pathogenesis in ichthyosis vulgaris and atopic dermatitis. In the granular layer, the proprotein profilaggrin is stored within the keratohyalin granules, where it is thought to be functionally inert. At the interface of the inner stratum corneum and the stratum granulosum, impaired aggregation of keratin filaments causes impaired excretion of lamellar bodies, with resultant impairment in barrier function. In the stratum corneum, filaggrin deficiency is associated with multiple structural changes, including decreased corneodesmosome density, decreased expression of tight-junction proteins, and — most important — impaired maturation and secretion of lamellar bodies. These changes may be mediated by increased pH within the residual cytoplasm of squames due to a lower concentration of acidic filaggrin breakdown products. All these changes contribute to impaired barrier function and increased ease of allergen presentation to epidermal dendritic cells. Finally, on the skin surface, decreased levels of natural moisturizing factor cause the skin to lose hydration and feel dry; reduced levels of urocanic acid and pyrrolidone carboxylic acid on the skin surface impair *Staphylococcus 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 epithelial inflammation and further barrier destruction.

persons[33,85] suggests an alternative mechanism for the barrier defect. Neutral or alkaline pH is optimal for several serine proteases.[86] Activation of kallikrein serine proteases has major downstream consequences, including activation of the plasminogen activator type 2 receptor–mediated block-ade of lamellar-body secretion.[87,88] Activation of serine proteases may also directly drive kallikrein 5–mediated Th2 inflammation, even in the absence of allergen priming.[89]

An elevation in the pH of the stratum corneum may lead to enhanced *S. aureus* adhesion and

multiplication.[31] 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.[31]

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[60]; thus, both genetic modifiers and environmental factors must be important. Epidemiologic studies suggest that early exposure to cats[90] may be an important environmental factor. In addition, the presence of elder siblings increases the risk of atopic dermatitis in *FLG* mutation carriers.[91] 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.[92]

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.[60] 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.[33] 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.[93] 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.[94] Interleukin-4 and interleukin-13 may inhibit filaggrin expression through down-regulation of keratinocyte differentiation by modulating the calcium-sensing protein S100A11.[95] Increased protease activity in the skin's inflammatory responses can lead to excessive degradation of filaggrin

by affecting the processing of profilaggrin.[20] 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.[96,97] The first approach is immunomodulation. Topical antiinflammatory agents have been shown to reverse the reduced filaggrin expression found in lesional atopic dermatitis,[98] 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 fool the cell into "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.[99]

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.

REFERENCES

1. Sandilands A, Sutherland C, Irvine AD, McLean WH. Filaggrin in the frontline: role in skin barrier function and disease. J Cell Sci 2009;122:1285-94.
2. Irvine AD, McLean WH. Breaking the (un)sound barrier: filaggrin is a major gene for atopic dermatitis. J Invest Dermatol 2006;126:1200-2.
3. Dale BA. Purification and characterization of a basic protein from the stratum corneum of mammalian epidermis. Biochim Biophys Acta 1977;491:193-204.
4. Steinert PM, Cantieri JS, Teller DC, Lonsdale-Eccles JD, Dale BA. Characterization of a class of cationic proteins that specifically interact with intermediate filaments. Proc Natl Acad Sci U S A 1981; 78:4097-101.
5. Fleckman P, Dale BA, Holbrook KA. Profilaggrin, a high-molecular-weight precursor of filaggrin in human epidermis and cultured keratinocytes. J Invest Dermatol 1985;85:507-12.
6. Smith FJ, Irvine AD, Terron-Kwiatkowski A, et al. Loss-of-function mutations in the gene encoding filaggrin cause ichthyosis vulgaris. Nat Genet 2006;38:337-42.
7. Presland RB, Haydock PV, Fleckman P, Nirunsuksiri W, Dale BA. 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;267:23772-81.
8. Gan SQ, McBride OW, Idler WW, Markova N, Steinert PM. Organization, structure, and polymorphisms of the human profilaggrin gene. Biochemistry 1990;29: 9432-40. [Erratum, Biochemistry 1991;30: 5814.]
9. Sandilands A, Terron-Kwiatkowski A, Hull PR, et al. Comprehensive analysis of the gene encoding filaggrin uncovers prevalent and rare mutations in ichthyosis vulgaris and atopic eczema. Nat Genet 2007; 39:650-4.
10. de Guzman Strong C, Conlan S, Deming CB, Cheng J, Sears KE, Segre JA. A milieu of regulatory elements in the epidermal differentiation complex syntenic block: implications for atopic dermatitis and psoriasis. Hum Mol Genet 2010;19:1453-60.
11. de Cid R, Riveira-Munoz E, Zeeuwen PL, et al. Deletion of the late cornified envelope LCE3B and LCE3C genes as a susceptibility factor for psoriasis. Nat Genet 2009;41:211-5.

12. Maestrini E, Monaco AP, McGrath JA, et al. A molecular defect in loricrin, the major component of the cornified cell envelope, underlies Vohwinkel's syndrome. Nat Genet 1996;13:70-7.
13. O'Regan GM, Sandilands A, McLean WH, Irvine AD. Filaggrin in atopic dermatitis. J Allergy Clin Immunol 2009;124: Suppl 2:R2-R6.
14. Dale BA, Holbrook KA, Kimball JR, Hoff M, Sun TT. Expression of epidermal keratins and filaggrin during human fetal skin development. J Cell Biol 1985;101: 1257-69.
15. Nemes Z, Steinert PM. Bricks and mortar of the epidermal barrier. Exp Mol Med 1999;31:5-19.
16. Candi E, Schmidt R, Melino G. The cornified envelope: a model of cell death in the skin. Nat Rev Mol Cell Biol 2005;6: 328-40.
17. List K, Szabo R, Wertz PW, et al. Loss of proteolytically processed filaggrin caused by epidermal deletion of Matriptase/MT-SP1. J Cell Biol 2003;163:901-10.
18. Leyvraz C, Charles RP, Rubera I, et al. The epidermal barrier function is dependent on the serine protease CAP1/Prss8. J Cell Biol 2005;170:487-96.
19. Deraison C, Bonnart C, Robin A, et al. KLK5 transgenic mice reproduce NS phenotype. J Invest Dermatol 2007;127:Suppl 2: S54. abstract.
20. Bonnart C, Deraison C, Lacroix M, et al. Elastase 2 is expressed in human and mouse epidermis and impairs skin barrier function in Netherton syndrome through filaggrin and lipid misprocessing. J Clin Invest 2010;120:871-82.
21. Matsui T, Miyamoto K, Kubo A, et al. SASPase regulates stratum corneum hydration through profilaggrin-to-filaggrin processing. EMBO Mol Med 2011;3:320-33.
22. Descargues P, Deraison C, Bonnart C, et al. Spink5-deficient mice mimic Netherton syndrome through degradation of desmoglein 1 by epidermal protease hyperactivity. Nat Genet 2005;37:56-65.
23. Takahashi M, Tezuka T, Katunuma N. Filaggrin linker segment peptide and cystatin alpha are parts of a complex of the cornified envelope of epidermis. Arch Biochem Biophys 1996;329:123-6.
24. Nachat R, Méchin MC, Takahara H, et al. Peptidylarginine deiminase isoforms 1-3 are expressed in the epidermis and involved in the deimination of K1 and filag-

grin. J Invest Dermatol 2005;124:384-93.
25. Scott IR, Harding CR. 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: 84-92.
26. Denecker G, Hoste E, Gilbert B, et al. Caspase-14 protects against epidermal UVB photodamage and water loss. Nat Cell Biol 2007;9:666-74.
27. Kamata Y, Taniguchi A, Yamamoto M, et al. Neutral cysteine protease bleomycin hydrolase is essential for the breakdown of deiminated filaggrin into amino acids. J Biol Chem 2009;284:12829-36.
28. Barresi C, Stremnitzer C, Mlitz V, 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: 188-94.
29. Walterscheid JP, Nghiem DX, Kazimi N, et al. Cis-urocanic acid, a sunlight-induced immunosuppressive factor, activates immune suppression via the 5-HT2A receptor. Proc Natl Acad Sci U S A 2006;103: 17420-5.
30. DeLapp NW, Dieckman DK. Gamma glutamyl peptidase: a novel enzyme from hairless mouse epidermis. J Invest Dermatol 1988;90:490-4.
31. Miajlovic H, Fallon PG, Irvine AD, Foster TJ. Effect of filaggrin breakdown products on growth of and protein expression by Staphylococcus aureus. J Allergy Clin Immunol 2011;126(6):1184.e3-1190.e3.
32. O'Regan GM, Kemperman PM, Sandilands A, et al. Raman profiles of the stratum corneum define 3 filaggrin genotype-determined atopic dermatitis endophenotypes. J Allergy Clin Immunol 2010;126:574-80.
33. Kezic S, O'Regan GM, Yau N, et al. Levels of filaggrin degradation products are influenced by both filaggrin genotype and atopic dermatitis severity. Allergy 2011;66:934-40.
34. Presland RB, Boggess D, Lewis SP, Hull C, Fleckman P, Sundberg JP. Loss of normal profilaggrin and filaggrin in flaky tail (ft/ft) mice: an animal model for the filaggrin-deficient skin disease ichthyosis vulgaris. J Invest Dermatol 2000;115:1072-81.
35. Mildner M, Jin J, Eckhart L, et al.

1325

Knockdown of filaggrin impairs diffusion barrier function and increases UV sensitivity in a human skin model. J Invest Dermatol 2010;130:2286-94.

36. Gruber R, Elias PM, Crumrine D, et al. Filaggrin genotype in ichthyosis vulgaris predicts abnormalities in epidermal structure and function. Am J Pathol 2011;178:2252-63.

37. de Juanes S, Epp N, Latzko S, et al. Development of an ichthyosiform phenotype in Alox12b-deficient mouse skin transplants. J Invest Dermatol 2009;129:1429-36.

38. Yanagi T, Akiyama M, Nishihara H, et al. Self-improvement of keratinocyte differentiation defects during skin maturation in ABCA12-deficient harlequin ichthyosis model mice. Am J Pathol 2010;177:106-18.

39. Hatano Y, Man MQ, Uchida Y, et al. Maintenance of an acidic stratum corneum prevents emergence of murine atopic dermatitis. J Invest Dermatol 2009;129:1824-35.

40. Wells RS, Kerr CB. Clinical features of autosomal dominant and sex-linked ichthyosis in an English population. BMJ 1966;1:947-50.

41. Mevorah B, Marazzi A, Frenk E. The prevalence of accentuated palmoplantar markings and keratosis pilaris in atopic dermatitis, autosomal dominant ichthyosis and control dermatological patients. Br J Dermatol 1985;112:679-85.

42. Sandilands A, O'Regan GM, Liao H, et al. Prevalent and rare mutations in the gene encoding filaggrin cause ichthyosis vulgaris and predispose individuals to atopic dermatitis. J Invest Dermatol 2006;126:1770-5.

43. Chen H, Common JE, Haines RL, et al. Wide spectrum of filaggrin-null mutations in atopic dermatitis highlights differences between Singaporean and European populations. Br J Dermatol 2011 March 24 (Epub ahead of print).

44. Liao H, Waters AJ, Goudie DR, et al. Filaggrin mutations are genetic modifying factors exacerbating X-linked ichthyosis. J Invest Dermatol 2007;127:2795-8.

45. Gruber R, Wilson NJ, Smith FJ, et al. Increased pachyonychia congenita severity in patients with concurrent keratin and filaggrin mutations. Br J Dermatol 2009;161:1391-5.

46. Shaw TE, Currie GP, Koudelka CW, Simpson EL. Eczema prevalence in the United States: data from the 2003 National Survey of Children's Health. J Invest Dermatol 2011;131:67-73.

47. Shamssain M. Trends in the prevalence and severity of asthma, rhinitis and atopic eczema in 6- to 7- and 13- to 14-yr-old children from the north-east of England. Pediatr Allergy Immunol 2007;18:149-53.

48. Kay J, Gawkrodger DJ, Mortimer MJ, Jaron AG. The prevalence of childhood atopic eczema in a general population. J Am Acad Dermatol 1994;30:35-9.

49. Lewis-Jones S. Quality of life and childhood atopic dermatitis: the misery of living with childhood eczema. Int J Clin Pract 2006;60:984-92.

50. Williams HC. Atopic dermatitis. N Engl J Med 2005;352:2314-24.

51. Mancini AJ, Kaulback K, Chamlin SL. The socioeconomic impact of atopic dermatitis in the United States: a systematic review. Pediatr Dermatol 2008;25:1-6.

52. Spergel JM, Paller AS. Atopic dermatitis and the atopic march. J Allergy Clin Immunol 2003;112:Suppl:S118-S127.

53. Palmer CN, Irvine AD, Terron-Kwiatkowski A, et al. Common loss-of-function variants of the epidermal barrier protein filaggrin are a major predisposing factor for atopic dermatitis. Nat Genet 2006;38:441-6.

54. Marenholz I, Nickel R, Rüschendorf F, et al. Filaggrin loss-of-function mutations predispose to phenotypes involved in the atopic march. J Allergy Clin Immunol 2006;118:866-71.

55. Ruether A, Stoll M, Schwarz T, Schreiber S, Fölster-Holst R. Filaggrin loss-of-function variant contributes to atopic dermatitis risk in the population of Northern Germany. Br J Dermatol 2006;155:1093-4.

56. Baurecht H, Irvine AD, Novak N, et al. Toward a major risk factor for atopic eczema: meta-analysis of filaggrin polymorphism data. J Allergy Clin Immunol 2007;120:1406-12.

57. Weidinger S, Illig T, Baurecht H, et al. Loss-of-function variations within the filaggrin gene predispose for atopic dermatitis with allergic sensitizations. J Allergy Clin Immunol 2006;118:214-9. [Errata, J Allergy Clin Immunol 2006;118:724, 922.]

58. Barker JN, Palmer CN, Zhao Y, et al. Null mutations in the filaggrin gene (FLG) determine major susceptibility to early-onset atopic dermatitis that persists into adulthood. J Invest Dermatol 2007;127:564-7.

59. van den Oord RA, Sheikh A. Filaggrin gene defects and risk of developing allergic sensitisation and allergic disorders: systematic review and meta-analysis. BMJ 2009;339:b2433.

60. Henderson J, Northstone K, Lee SP, et al. The burden of disease associated with filaggrin mutations: a population-based, longitudinal birth cohort study. J Allergy Clin Immunol 2008;121(4):872.e9-877.e9.

61. Gao PS, Rafaels NM, Hand T, et al. Filaggrin mutations that confer risk of atopic dermatitis confer greater risk for eczema herpeticum. J Allergy Clin Immunol 2009;124:507-13.

62. Irvine AD. Fleshing out filaggrin phenotypes. J Invest Dermatol 2007;127:504-7.

63. de Jongh CM, John SM, Bruynzeel DP, et al. Cytokine gene polymorphisms and susceptibility to chronic irritant contact dermatitis. Contact Dermatitis 2008;58:269-77.

64. Novak N, Baurecht H, Schafer T, et al. Loss-of-function mutations in the filaggrin gene and allergic contact sensitization to nickel. J Invest Dermatol 2008;128:1430-5.

65. Betz RC, Pforr J, Flaquer A, 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;127:2539-43.

66. Weidinger S, O'Sullivan M, Illig T, et al. Filaggrin mutations, atopic eczema, hay fever, and asthma in children. J Allergy Clin Immunol 2008;121(5):1203.e1-1209.e1.

67. Ying S, Meng Q, Corrigan CJ, Lee TH. Lack of filaggrin expression in the human bronchial mucosa. J Allergy Clin Immunol 2006;118:1386-8.

68. McLean WH, Palmer CN, Henderson J, Kabesch M, Weidinger S, Irvine AD. Filaggrin variants confer susceptibility to asthma. J Allergy Clin Immunol 2008;121:1294-5.

69. Ziegler SF, Artis D. Sensing the outside world: TSLP regulates barrier immunity. Nat Immunol 2010;11:289-93.

70. Palmer CN, Ismail T, Lee SP, et al. Filaggrin null mutations are associated with increased asthma severity in children and young adults. J Allergy Clin Immunol 2007;120:64-8.

71. Illi S, von Mutius E, Lau 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;113:925-31.

72. Sicherer SH, Furlong TJ, Maes HH, Desnick RJ, Sampson HA, Gelb BD. Genetics of peanut allergy: a twin study. J Allergy Clin Immunol 2000;106:53-6.

73. Brown SJ, Asai Y, Cordell HJ, et al. Loss-of-function variants in the filaggrin gene are a significant risk factor for peanut allergy. J Allergy Clin Immunol 2011;127:661-7.

74. Boguniewicz M, Leung DYM. Recent insights into atopic dermatitis and implications for management of infectious complications. J Allergy Clin Immunol 2010;125:4-13.

75. Bieber T. Atopic dermatitis. N Engl J Med 2008;358:1483-94.

76. Elias PM, Feingold KR. Does the tail wag the dog? Role of the barrier in the pathogenesis of inflammatory dermatoses and therapeutic implications. Arch Dermatol 2001;137:1079-81.

77. Werner Y, Lindberg M. Transepidermal water loss in dry and clinically normal skin in patients with atopic dermatitis. Acta Derm Venereol 1985;65:102-5.

78. Fartasch M, Diepgen TL. The barrier function in atopic dry skin: disturbance of membrane-coating granule exocytosis and formation of epidermal lipids? Acta Derm Venereol Suppl (Stockh) 1992;176:26-31.

79. Rodríguez E, Illig T, Weidinger S. Filaggrin loss-of-function mutations and association with allergic diseases. Pharmacogenomics 2008;9:399-413.

80. Scharschmidt TC, Man MQ, Hatano Y, et al. Filaggrin deficiency confers a paracellular barrier abnormality that reduces inflammatory thresholds to irritants and haptens. J Allergy Clin Immunol 2009; 124:496-506.

81. Oyoshi MK, Murphy GF, Geha RS. Filaggrin-deficient mice exhibit TH17-dominated skin inflammation and permissiveness to epicutaneous sensitization with protein antigen. J Allergy Clin Immunol 2009;124:485-93.

82. Moniaga CS, Egawa G, Kawasaki H, et al. Flaky tail mouse denotes human atopic dermatitis in the steady state and by topical application with Dermatophagoides pteronyssinus extract. Am J Pathol 2010;176:2385-93.

83. De Benedetto A, Rafaels NM, McGirt LY, et al. Tight junction defects in patients with atopic dermatitis. J Allergy Clin Immunol 2011;127:773-86.

84. Krien PM, Kermici M. Evidence for the existence of a self-regulated enzymatic process within the human stratum corneum — an unexpected role for urocanic acid. J Invest Dermatol 2000;115:414-20.

85. Jungersted JM, Scheer H, Mempel M, et al. Stratum corneum lipids, skin barrier function and filaggrin mutations in patients with atopic eczema. Allergy 2010; 65:911-8.

86. Brattsand M, Stefansson K, Lundh C, Haasum Y, Egelrud T. A proteolytic cascade of kallikreins in the stratum corneum. J Invest Dermatol 2005;124:198-203.

87. Hachem JP, Man MQ, Crumrine D, et al. Sustained serine proteases activity by prolonged increase in pH leads to degradation of lipid processing enzymes and profound alterations of barrier function and stratum corneum integrity. J Invest Dermatol 2005;125:510-20.

88. Demerjian M, Hachem JP, Tschachler E, et al. Acute modulations in permeability barrier function regulate epidermal cornification: role of caspase-14 and the protease-activated receptor type 2. Am J Pathol 2008;172:86-97.

89. Briot A, Deraison C, Lacroix M, et al. Kallikrein 5 induces atopic dermatitis-like lesions through PAR2-mediated thymic stromal lymphopoietin expression in Netherton syndrome. J Exp Med 2009;206:1135-47.

90. Bisgaard H, Simpson A, Palmer CN, et al. Gene-environment interaction in the onset of eczema in infancy: filaggrin loss-of-function mutations enhanced by neonatal cat exposure. PLoS Med 2008;5(6): e131.

91. Cramer C, Link E, Horster M, et al. Elder siblings enhance the effect of filaggrin mutations on childhood eczema: results from the 2 birth cohort studies LISAplus and GINIplus. J Allergy Clin Immunol 2010;125(6):1254.e5-1260.e5.

92. Weidinger S, Baurecht H, Wagenpfeil S, et al. Analysis of the individual and aggregate genetic contributions of previously identified serine peptidase inhibitor Kazal type 5 (SPINK5), kallikrein-related peptidase 7 (KLK7), and filaggrin (FLG) polymorphisms to eczema risk. J Allergy Clin Immunol 2008;122(3):560.e4-568.e4. [Erratum, J Allergy Clin Immunol 2008;122:976.]

93. Howell MD, Kim BE, Gao P, et al. Cytokine modulation of atopic dermatitis filaggrin skin expression. J Allergy Clin Immunol 2007;120:150-5.

94. Sehra S, Yao Y, Howell MD, et al. IL-4 regulates skin homeostasis and the predisposition toward allergic skin inflammation. J Immunol 2010;184:3186-90.

95. Howell MD, Fairchild HR, Kim BE, et al. Th2 cytokines act on S100/A11 to down-regulate keratinocyte differentiation. J Invest Dermatol 2008;128:2248-58.

96. Elias PM, Wakefield JS. Therapeutic implications of a barrier-based pathogenesis of atopic dermatitis. Clin Rev Allergy Immunol 2010 December 21 (Epub ahead of print).

97. Lane EB, McLean I. Broken bricks and cracked mortar: epidermal diseases resulting from genetic abnormalities. Drug Discov Today Dis Mech 2008;5(1):e93-e101.

98. Jensen JM, Pfeiffer S, Witt M, et al. Different effects of pimecrolimus and betamethasone on the skin barrier in patients with atopic dermatitis. J Allergy Clin Immunol 2009;124:Suppl 2:R19-R28.

99. Rowe SM, Clancy JP. Pharmaceuticals targeting nonsense mutations in genetic diseases: progress in development. BioDrugs 2009;23:165-74.

Copyright © 2011 Massachusetts Medical Society.

POSTING PRESENTATIONS FROM MEDICAL MEETINGS ONLINE

Online posting of an audio or video recording of an oral presentation at a medical meeting, with selected slides from the presentation, is not considered prior publication. Authors should feel free to call or send e-mail to the *Journal*'s Editorial Offices if there are any questions about this policy.