

The role of filaggrin loss-of-function mutations in atopic dermatitis

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Purpose of review

To provide a comprehensive summary of recent genetic advances as they relate to the pathogenesis of atopic dermatitis.

Recent findings

Atopic dermatitis is a common inflammatory skin disease with a complex cause, resulting from an elaborate interplay between environmental, immunological and genetic factors. The disease is often the prelude to an atopic diathesis that includes asthma and other allergic diseases. The identification of mutations in the barrier protein filaggrin as conferring major susceptibility to atopic dermatitis and atopic dermatitis related asthma has reconfigured our understanding of disease mechanisms and highlights the importance of epidermal barrier disruption as a primary event in the disease.

Summary

In this review we highlight recent advances in our understanding of how filaggrin might influence the environmental-immune interface, impacting disease penetrance, severity and trajectory, and the implications for both research and therapeutics in this field. Focusing on the downstream biological consequences of altered filaggrin expression and the sequence of immunological and environmental triggers that ensue will provide the rationale for targeted therapeutics capable of restoring or preventing disruption of barrier function.

Keywords

atopic dermatitis, barrier function, eczema, filaggrin, ichthyosis vulgaris, natural moisturizing factor, pH, proteases, *Staphylococcus aureus*

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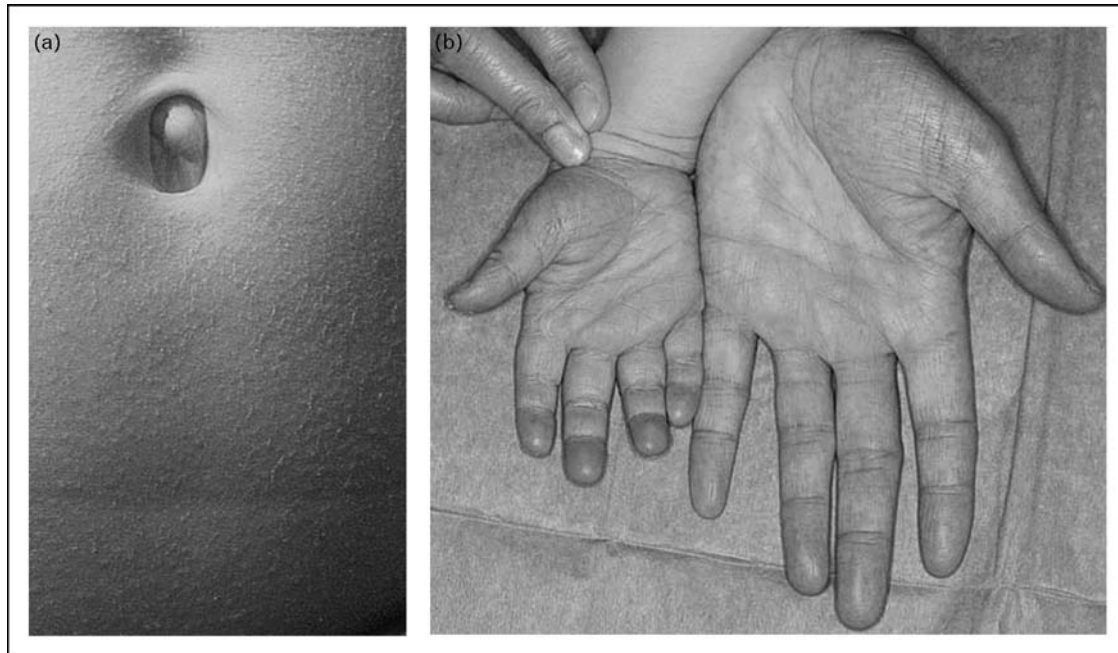
Introduction

Filaggrin, or filament-aggregating protein (gene symbol: *FLG*), is a highly abundant epithelial barrier protein that has recently emerged as a central component in the pathogenesis of atopic dermatitis and progression to the atopic phenotype [1–5]. *FLG* was proposed as a candidate gene for atopic dermatitis in 2006, following its identification as the causal mutation in ichthyosis vulgaris [6]. Ichthyosis vulgaris (OMIM 146700) is the most common of the inherited ichthyotic disorders, estimated to affect one in 250 individuals, and is characterized by generalized fine white scale, palmoplantar hyperlinearity and keratosis pilaris (Fig. 1) [7]. For many years much indirect evidence pointed to mutations in *FLG* as causative, however, the large and impressively repetitive gene sequence hampered amplification [8–10]. The identification of two recurrent mutations, R501X and 2282del4 within exon 3 of *FLG* by Smith *et al.* using long-range sequencing and multiple alignment techniques convincingly closed the chapter on one of the few remaining single gene inherited skin disorders to be

identified [6]. The unexpected finding of a combined allele frequency of almost 10% in individuals of European ancestry, and the known association of ichthyosis vulgaris with atopy, pointed to the intriguing possibility that *FLG* might be associated with more complex-disease states such as atopic dermatitis [11]. This association has now been unambiguously established in a large series of replication studies, making this one of the most robust gene associations so far identified in complex trait genetics [12,13*,14–19]. Over the past 2 years, the number of mutations identified has increased steadily, with a spectrum of mutations unfolding worldwide, adding considerable weight to the role of filaggrin in the atopic story globally [20*,21–24].

Filaggrin biochemistry

Insight into the contributory role of *FLG* to the atopic diathesis may be gleaned from its role in establishing barrier homeostasis, and the putative biological effects of its metabolites. The protective barrier function provided by stratified squamous epithelia resides in the cornified

Figure 1 Ichthyosis vulgaris is characterized by (a) diffuse fine scale and (b) marked palmar hyperlinearity

envelope, a structure synthesized at late stages of keratinocyte differentiation [25]. The formation of this barrier is a dynamic and precisely regulated series of biochemical events that is initiated by the sequential expression and crosslinking of structural proteins [26]. Many of these proteins cluster within a locus encoded for on chromosome 1q21, known as the epidermal differentiation complex (EDC) and include proteins such as loricrin, involucrin, small proline-rich proteins (SPRs), late envelope proteins (LEPs) and the S100 calcium-binding proteins, of which *FLG* is a founding member [27]. During formation of the cornified envelope, the giant inactive proprotein of filaggrin, profilaggrin, is dephosphorylated and proteolytically cleaved by serine proteases to release multiple copies of the functional filaggrin repeat peptide units [28]. These liberated filaggrin monomers bind to and collapse the keratin cytoskeleton and other intermediate filaments, acting as a scaffold for the subsequent reinforcement steps of cornified envelope assembly [29]. This structure is subsequently chemically crosslinked by transglutaminases and ultimately surrounded by a lipid bi-layer, forming a critical interface barrier. In response to deficiency, injury or other environmental triggers, proteins forming the cornified envelope may be upregulated in an effort to compensate and maintain an effective barrier [30,31]. Additionally, compensatory reduction in epidermal protease activity has been shown to improve phenotype in murine models. [32] Functional polymorphisms of these proteins or compensatory upregulation of proteases may, thus, influence atopic dermatitis severity and chronicity, adding to the complexity of phenotype and dissection of atopic mechanisms. Genetically determined modifiers of

the atopic phenotype may also include the copy number of filaggrin repeat units. There are 10 homologous *FLG* polypeptide units; however, the number of filaggrin-repeat units in the population is variable, consisting of 10, 11 or 12 units. This genetic polymorphism reflects tandem duplications of filaggrin repeats eight or 10 or both and may be an additional modifier of disease phenotype in heterozygotes that carry longer sized variants on the unaffected allele [2,33].

Once liberated, filaggrin is progressively degraded by post-translational modification enzymes [including peptidylarginine deiminase (PAD 1 and 3 isoforms)] into a pool of hydrophilic amino acids including urocanic acid, pyrrolidone carboxylic acid and alanine [34]. This combined pool of amino acids, their metabolites and various ions make up what is known as the natural moisturising factor (NMF). Collectively, these osmotically active molecules play a key role in maintaining hydration of the stratum corneum [35]. NMF levels have been shown to correlate with *FLG* null allele status, suggesting a gene-dose effect, and may therefore directly contribute to the dry skin phenotype seen in both ichthyosis vulgaris and atopic dermatitis [36].

Expression of *FLG* and subsequent activation of hydrolysis of *FLG* into NMF are additionally determined by the properties of the microenvironment, including local pH, relative humidity and protease activity [37]. The *FLG* breakdown products that make up NMF in turn play a critical role in the physiological maintenance of the pH gradient of the skin [38,39]. This acid mantle regulates

key biochemical events including protease activity, barrier permeability and cutaneous antimicrobial defence [40]. Filaggrin thus has complex interrelated functions, with strategic roles in establishing structural and chemical barrier function, hydration of the skin and maintaining epidermal homeostasis in the face of continuous trans-formation.

Filaggrin mutations and atopic dermatitis

Since the initial report of mutations, sequencing strategies have identified a total of 32 *FLG* mutation populations worldwide [41]. To date, the number of mutations in European populations is 16, each predicting nonsense or out-of-frame deletion mutations, of which six are prevalent and 10 are of low frequency. In Asian populations, an additional 14 mutations, of which five are prevalent and nine are of low frequency, have been identified. With only two exceptions, both involving single cases, the known filaggrin mutations are race-specific. The high-allele frequency and identification of functional polymorphisms outside of European populations supports the hypothesis that these large-effect mutations have resulted from evolutionarily driven adaptation.

Meta-analysis of nine comparable studies of patients with atopic dermatitis has estimated for the combined null phenotype of the two initial mutations, R501X and 2282del4, an odds ratio of 4.09 [95% confidence interval (CI) 2.64–6.33] from case–control studies and an odds ratio of 2.06 [95% CI 1.76–2.42] from family studies [42]. Between 14 and 56% of individuals with atopic dermatitis carry one or more *FLG*-null allele, conferring a 1.2–13 times increased risk of developing atopic dermatitis; with higher risk in cohorts with persistent, moderate or severe eczema and those analysed for an expanded number of *FLG* mutations [2,43]. These results have also been replicated in population-based cohorts, with an associated three-fold increased risk of developing atopic dermatitis in the largest cohort to date, ($n = 6971$), in which the two common *FLG* mutations R501X and 2282del4 were analysed, with a calculated odds ratio of 3.12 (95% CI 2.33–4.17, $P = 2.5 \times 10^{-14}$) [44]. Significantly, these mutations have also been shown to be highly associated with allergen sensitization and the subsequent development of asthma associated with eczema, with emerging evidence for increased severity of asthma [45]. As *FLG* is not expressed in bronchial mucosa, this strongly implicates transcutaneous sensitization as a major contributor to the development of systemic atopic responses, linking primary skin disorders to systemic allergies [46]. This paradigm of defective barrier function driving allergic sensitization is further supported by the association of *FLG* mutations, and atopic dermatitis is associated with elevated serum IgE levels [47*]. These observations have

wide-ranging implications for our understanding of disease mechanisms and raises many more questions.

Possible mechanisms of disease

The downstream consequences of altered filaggrin expression may provide unifying biochemical answers to the aberrant regulatory events that define atopic dermatitis. These may include altered cutaneous microflora, triggering of the innate immune response and increased protease activity. Increased pH facilitates the growth of *Staphylococcus aureus*, which colonizes the skin of over 90% of eczema patients, up to 65% of which are capable of producing exotoxins with superantigenic properties [48,49]. The virulence factors produced by *S. aureus* have a wide range of biological properties including disruption of the epithelial barrier, inhibition of opsonization, interference with neutrophil chemotaxis, cytolysis of neutrophils and inactivation and downregulation of antimicrobial peptides including cathelicidin LL-37, the human β -defensins HBD-1, HBD-2 and HBD-3; and dermicidin [51,60]. Exposure of a naive immune system to *S. aureus* superantigens may trigger and establish a permanent Th2 immune response through activation and amplification of innate immune responses [52,53]. IL-31, a novel Th2 cytokine, is significantly overexpressed in pruritic atopic lesions, and staphylococcal superantigen rapidly induces IL-31 expression in atopic individuals, establishing a link between staphylococcal colonization and pruritus induction in patients with atopic dermatitis [54,55]. In turn, activated skin infiltrating T cell may become new source of IL-31, thereby amplifying atopic skin inflammation and pruritus [56]. Furthermore, these strains are known to have high levels of proteolytic activity, contributing to further disruption of barrier function [57]. Neutralizing stratum corneum pH has also been shown to independently facilitate excessive protease activity and reduce the activity of key lipid-processing enzymes resulting in the formation of defective lamellar membranes, and thus a disrupted permeability barrier [58]. In addition, keratinocytes in atopic skin produce high levels of the IL-7-like stromal lymphopoietin that signals dendritic cells to drive Th2 polarization [59]. Recent evidence also indicates that induction of filaggrin is significantly inhibited by the presence of IL-4 and IL-13, suggesting that skin barrier function may be altered by the atopic immune response in patients with eczema who do not carry *FLG* mutations [50].

Epistatic mechanisms

Genetic influences have been postulated to modify *FLG* expression. Maternally derived polymorphisms in the serine protease inhibitor serine peptidase inhibitor, Kazal type 5 (*SPINK5*) (particularly Glu420Lys), have been

shown to modify the risk of developing eczema, asthma and IgE, suggesting a pathway may lie in altered expression of environmental proteases, although the effect size has been shown to be small [61]. Gain of function polymorphisms in the *kallikrein 7* gene (*KLK7*) encoding the protease stratum corneum chemotryptic enzyme (SSCE), have also been reported to adversely affect barrier function, possibly through the proteolytic processing of profilaggrin [62]. More recently, however, two large European cohort studies failed to find an association between the *KLK7* insertion and atopic dermatitis. Furthermore, no evidence was found to support epistatic effects between *KLK7* or *SPINK5* and *FLG* mutations [63,64].

Abnormal barrier genes such as *FLG* mutants may have a permissive effect in the early inflammatory effects that characterize atopic dermatitis. However, any paradigm put forward to explain the increase in atopic disease must take into account that genetic factors modifying susceptibility remain largely stable, therefore, the increased prevalence must be related to dose effect with environmental or secondary immunobiological factors [65]. Prolonged exposure to a reduced environmental humidity, as occurs in air-conditioned or radiantly heated homes, may alter filaggrin proteolysis, contributing to the dramatic increase in atopic dermatitis in the western world [37]. Other potential modifiers or sensitizers include increased exposure to high-molecular-weight allergens in pollens, house dust mite products, microbes and food, driving dendritic cells to further enhance Th2 polarization in the context of a defective barrier [66].

Conclusion

Recent insights into the genetic and immunobiological mechanisms of atopic dermatitis opens new challenging research goals: targeted therapies directed specifically at the molecular defects in the stratum corneum, impacting on *FLG* expression and prevention of the signalling cascades that lead to inflammation and systemic sensitization. Potential pharmacogenetic targets for established atopic disease include screening for compounds capable of inducing filaggrin expression. Understanding the pathomechanisms of atopic disease and directed intervention in susceptible individuals may alter disease trajectory and reverse the prevalence of a disease that is an increasing burden to global healthcare systems.

References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

- of special interest
- of outstanding interest

Additional references related to this topic can also be found in the Current World Literature section in this issue (p. 491).

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