Review Article

Skin barrier genetics: filaggrin and the dermatologist

皮膚屏障遺傳學：聚絲蛋白和皮膚科醫生的千絲萬縷

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Many dermatologists may question the relevance of genetics to their every day clinical practice. While this might be true for the rarely encountered genodermatoses such as epidermolysis bullosa or xeroderma pigmentosum, it is becoming clear that for some common dermatoses, genetics is in fact at the core of trying to develop new and better treatments for patients. One emerging example of this is the gene encoding the skin protein filaggrin, defects in which are now known to cause ichthyosis vulgaris and which also constitute a major risk factor for atopic dermatitis and other skin and systemic allergies. By understanding how filaggrin gene mutations disrupt the skin barrier as well as their downstream consequences on skin function and inflammation, new insights into skin biology and disease mechanisms can be established. Moreover, targeting new therapies to boost filaggrin expression in skin is destined to change the way we manage a range of conditions from mild xerosis to severe forms of atopic dermatitis.

一些皮膚科醫生可能會質疑遺傳學與他們日常臨床診治的關聯。就罕見的皮膚遺傳病如表皮分解性水皰症或著色性乾皮症來說實不爲過；但反之常見的皮膚病，遺傳學的重要性則越來越清楚易見，並將成爲發展新療法及改善病人治療的核心。其中新近的例子便是皮膚蛋白之聚絲蛋白的編碼基因，現今所知其缺陷可造成尋常性魚鱗癬，亦是異位性皮膚炎和其他皮膚及系統性感敏症的主要風險因子。我們若能明白聚絲蛋白的基因突變是怎樣擾亂皮膚屏障，並了解其對皮膚功能和發炎所產生的後果，便能在皮膚生物學及致病機理上建立一些新視野。同時，集中研發促進皮膚聚絲蛋白形成的新療法，定能爲一系列由輕微的乾燥症至嚴重的異位性皮膚炎的皮膚病治療，打造出一個新天地。

Keywords: Allergy, atopy, dermatitis, eczema, epidermis, epidermal differentiation, ichthyosis

關鍵詞：過敏，遺傳性過敏症，皮炎，濕疹，表皮，表皮細胞分化，魚鱗病

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Introduction

One of the main functions of human skin is to form an effective mechanical barrier against the external environment.[1,2] This involves the maturation and death of epidermal keratinocytes as well as the assembly of a complex network of differentially and spatially expressed proteins,

glycoproteins and lipids into the keratinocyte cell membrane and surrounding extracellular space.[3] A key component in forming an effective skin barrier is the protein (pro) filaggrin, the major component of keratohyalin granules in the granular cell layer.

What is filaggrin?

In the granular cell layer of the epidermis a number of cellular events occur: lamellar granules (Golgi-derived membrane-bound organelles that contain lipid) are extruded, apoptosis is initiated, the nuclei and other cellular contents susceptible to proteolysis are destroyed, and the potential for protein synthesis is lost.[3] Within this part of the epidermis, the main keratinocyte proteins are keratin and filaggrin, which together contribute approximately 80-90% of the mass of the epidermis. Filaggrin is initially synthesized as profilaggrin, a 500-kDa highly phosphorylated, histidine-rich polypeptide, that consists of an amino-terminal S100 calcium-binding domain, a downstream B-domain and a carboxy-terminal domain, as well as a central region comprising a series of repeat units of filaggrin (Figure 1). Profilaggrin is the main component of keratohyalin granules. During the post-translational processing of profilaggrin, the individual filaggrin polypeptides, each approximately 35 kDa, are proteolytically released.[4] These sub-units are then dephosphorylated, a process that assists keratin filament aggregation and explains the origin of the name 'filaggrin' (**fil**ament **aggr**egating prote**in**). Filaggrin has a vital role in the initial formation of flattened keratinocytes in the process of forming corneocytes and an intact skin barrier. In the stratum corneum, filaggrin proteolysis occurs. Multiple enzymes can be involved in this process, including caspase 14.[5] Filaggrin proteolysis releases histidine which is then deaminated to form trans-urocanic acid which is then converted to cis-urocanic acid by ultraviolet irradiation. Glutamic acid released from filaggrin is converted into pyroglutamic acid which may function as a natural moisturising substance (Figure 2).

Filaggrin and dry skin

The gene that encodes profilaggrin, *FLG*, comprises three exons, the initiation codon for translation being located in exon 2, and the bulk of the profilaggrin protein is encoded by exon 3. *FLG* resides on human chromosome 1q21 within the epidermal-differentiation complex, a region that also harbours genes for numerous other proteins that are expressed during terminal differentiation of keratinocytes, several of which become cross-linked enzymatically into the cornified cell envelope. The *FLG* gene encodes 10 highly homologous and only slightly genetically different filaggrin polypeptide units. The considerable DNA sequence overlap in these repeat units accounts for why many investigators have found the *FLG* gene very difficult to amplify by polymerase chain reaction. It is known that different individuals can have 10, 11 or 12 filaggrin repeat units, with the additional units consisting of repeats of units 8 or 10 or both of these.[6] The precise but variable number of filaggrin-repeat units encoded by each profilaggrin allele is genetically determined. These are not "mutations" *per se*, but represent allelic variations among different individuals globally. Filaggrin is a natural moisturiser, containing several hydrophilic amino acids capable of retaining water, and individuals with only 10 filaggrin repeats tend to have dryer skin than those with 11 or 12 repeats.[6] Thus for patients in the clinic with dry skin but no clear dermatological disease, one explanation maybe that such individuals could have fewer filaggrin repeats.

Filaggrin mutations cause ichthyosis vulgaris

Ichthyosis vulgaris is a common heritable skin disorder characterized by dry scaly skin that affects approximately 1 in 250 individuals. The diagnosis is usually a clinical one, although histopathological demonstration of abnormal or absent keratohyalin granules can aid diagnosis.[7] In support of the *FLG* gene being the candidate gene for ichthyosis vulgaris, several pedigrees have been genetically mapped to the epidermal differentiation complex on chromosome 1q21.[8] In 2006, researchers in Dundee University in Scotland were finally able
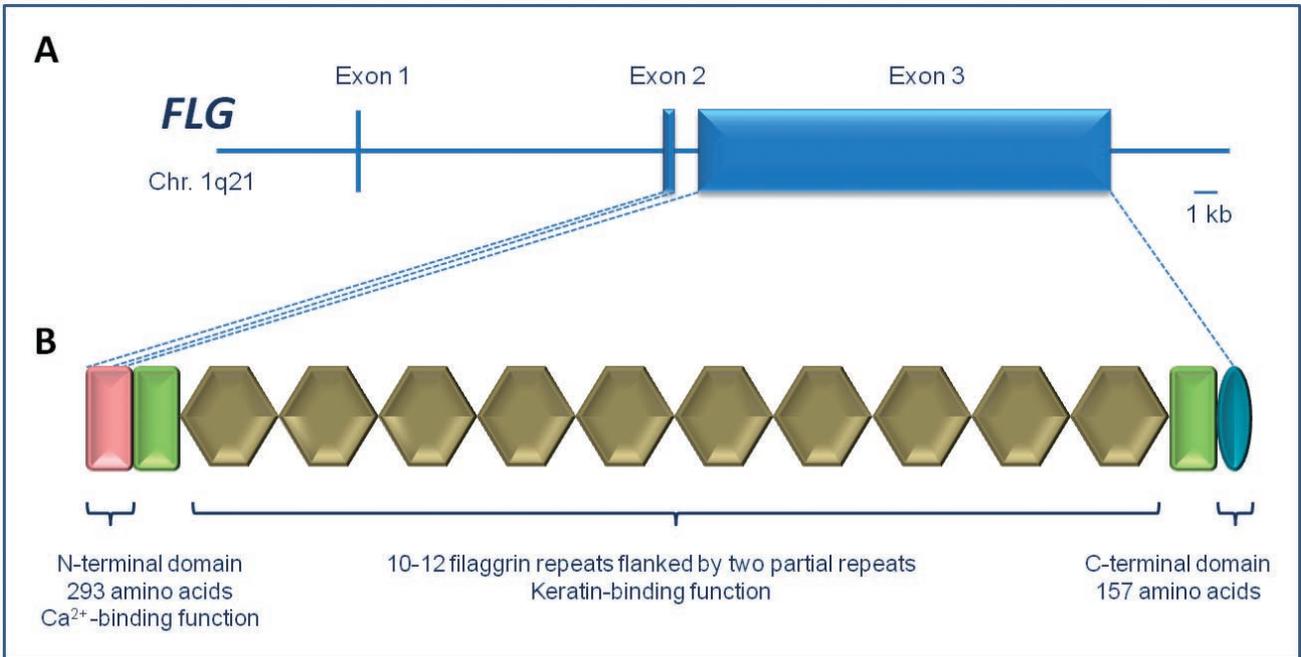
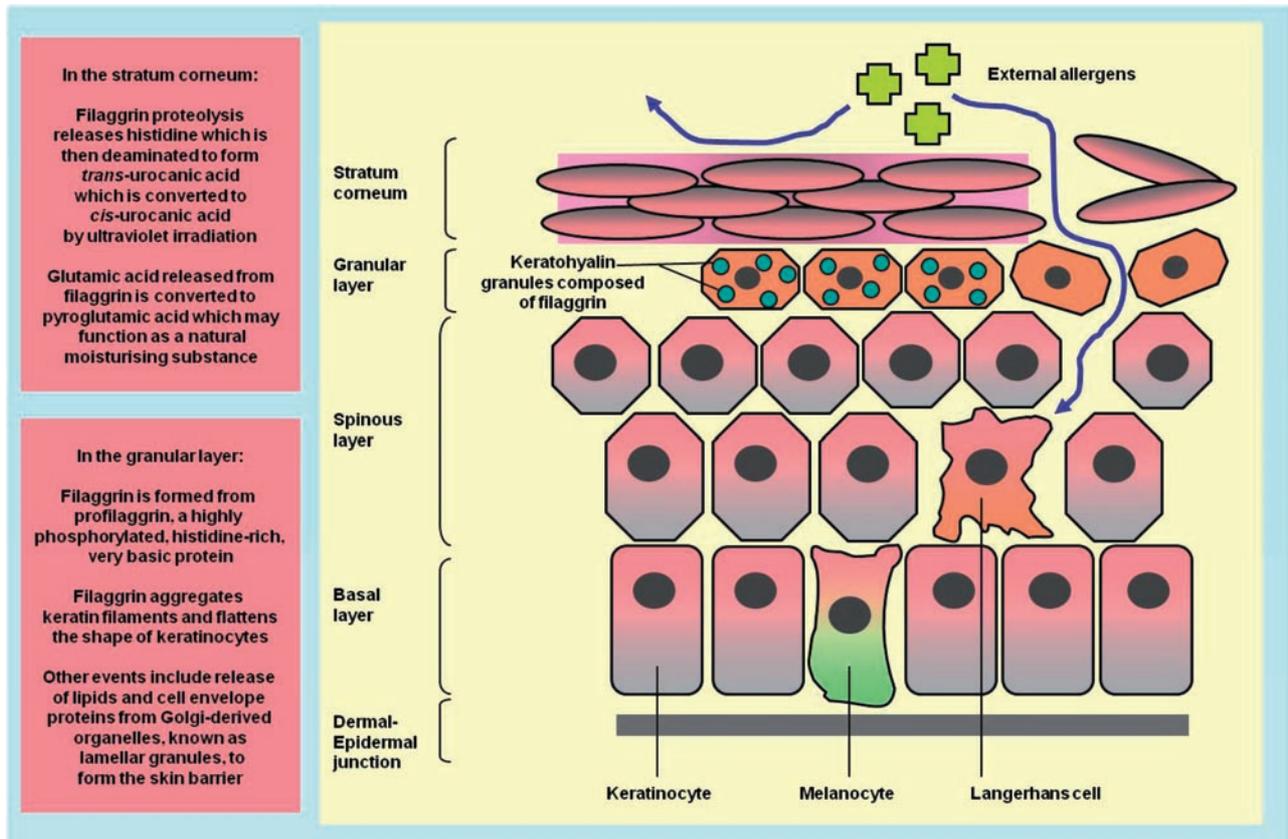**Figure 1.** The filaggrin gene (A) and protein (B).

Figure 2. The role of filaggrin in the granular cell layer and the stratum corneum. Loss of filaggrin expression (right side of picture), can lead to skin barrier disruption and penetration of allergens.

to report what had been long suspected: that ichthyosis vulgaris is caused by loss-of-function mutations in the *FLG* gene.[9] In particular, two common premature termination codon-causing mutations, pArg501X and c.2282del4, were identified in 15 white northern European pedigrees studied initially.[9] These investigators were able to demonstrate that ichthyosis vulgaris is a semi-dominant condition with incomplete penetrance. The heterozygotes displayed mild scaling or no phenotype at all, whereas homozygotes or compound heterozygotes typically had a severe form of ichthyosis vulgaris manifesting with dry, scaly skin with altered skin barrier function. The penetrance of the gene effect was approximately 90% for individuals with two *FLG* mutations and 60% for those with a single *FLG* mutation. This means that although *FLG* mutations represent the cause of ichthyosis vulgaris, not every individual who has *FLG* mutations will manifest the disease, and that other genes or environmental influences are relevant to the phenotypic expression.[10]

Filaggrin mutations predispose to atopic dermatitis

A remarkable observation from the initial *FLG* gene mutation report was that the two null mutations, p.Arg501X and c.2282del4, were extremely common in the general population, occurring in approximately 10% of European individuals.[9] To dermatologists, aware that from a clinical perspective ichthyosis vulgaris and atopic dermatitis often co-exist in the same patient, a natural next question was to wonder whether *FLG* mutations might also be relevant to the genetics of atopic dermatitis. Subsequent studies were then able to show that atopic dermatitis was manifested in heterozygous carriers of these *FLG* mutations with a relative risk (odds ratio) for atopic dermatitis of 3.1, thus implying a causal relationship.[11] This initial demonstration of *FLG* mutations in atopic dermatitis has now been replicated in a number of populations, including several European and Asian countries.[12-14] Individuals with one FLG mutation (heterozygotes) have a six-fold increased risk of developing atopic dermatitis; for those with

two FLG mutations (homozygotes/compound heterozygotes) the risk increases to 150-fold. Thus FLG mutations represent a major risk factor for atopic dermatitis. In terms of what this means in the clinic, it is likely that approximately 50% of all (European) patients seen with atopic dermatitis are likely to harbour at least one *FLG* gene mutation, especially in individuals with more severe disease. For the other 50% of cases, changes in the *FLG* gene may not be a primary genetic risk factor although inflammation in the skin can lead to a secondary reduction in filaggrin protein and thus filaggrin may still be implicated in the pathophysiology of atopic dermatitis in such cases.

The global spectrum of filaggrin mutations

The *FLG* mutations p.Arg501X and c.2282del4 are shared across many European countries but studies in Asia have reported more variation among populations in Japan, China, Singapore, Taiwan and Bangladesh.[13] Although the prevalence of atopic dermatitis is similar in most countries, the overall incidence of *FLG* gene mutations in Asia seems to be slightly less than in Europe (especially northern Europe) and there are fewer recurrent mutations, i.e. the mutations that are present in Asia are different and more diverse.[9,13] Moreover, while approximately 50% of Europeans with atopic dermatitis harbour at least one *FLG* mutation, in Asia this figure seems to be closer to 20%.[13] Common to all populations, however, are some clinical clues to the presence of underlying *FLG* gene mutations. Although an association has been established between *FLG* mutations and keratosis pilaris, perhaps one even more useful clinical sign is the presence of hyperlinearity of the palms – specifically an increased number of vertical or horizontal lines (or a combination of both) on the thenar eminences. Examination for this physical sign is recommended in the evaluation of all patients with atopic dermatitis or dry skin.[15] Splitting patients with atopic dermatitis into *FLG* gene mutation-positive and -negative subgroups may become a useful thing to do, both for targeting future therapies as well as for the identification of other genetic and environmental events that could

also trigger atopic dermatitis. At the moment, screening for *FLG* mutations is restricted to a few research laboratories worldwide, although more "routine" and easily accessible screening is anticipated in the near future.

Filaggrin mutations, other atopic diseases and allergy

Atopic dermatitis is part of an atopic triad, often occurring in association with asthma and allergic rhinitis. In individuals with atopic dermatitis who have *FLG* mutations, there is also an association with susceptibility to asthma.[16,17] Of note, however, *FLG* mutations are only associated with asthma in combination with atopic dermatitis and not with asthma alone. *FLG* mutations have also been shown to be associated with greater atopic asthma severity.[17] Given that profilaggrin is not expressed in pulmonary epithelia, these findings raise the intriguing possibility that asthma in individuals with atopic dermatitis is secondary to sensitisation via a primarily defective epidermal barrier, which allows allergens to enter the skin and make contact with cutaneous antigen-presenting cells. The possibility that a defective skin barrier due to *FLG* mutations is linked to systemic allergy is further supported by data demonstrating an association between *FLG* mutations and atopic dermatitis associated with high serum immunoglobulin E levels and allergic sensitisation.[18] Thus, a number of systemic allergies may be initiated through a defective skin barrier due directly to *FLG* gene mutations, observations that provide fascinating new insight into potential mechanisms of allergic disease as well as the "atopic march". *FLG* mutations have also been identified as a risk factor for contact dermatitis and for peanut allergy.[19,20] The common occurrence of *FLG* mutations in the population means that although they are not directly implicated in diseases such as psoriasis, acne vulgaris or lichen planus, the coincidental presence of *FLG* mutations in patients with these skin conditions could serve as disease modifiers and have implications for optimal patient therapy.[21] An overview of the clinical associations of *FLG* mutations is shown in Figure 3.
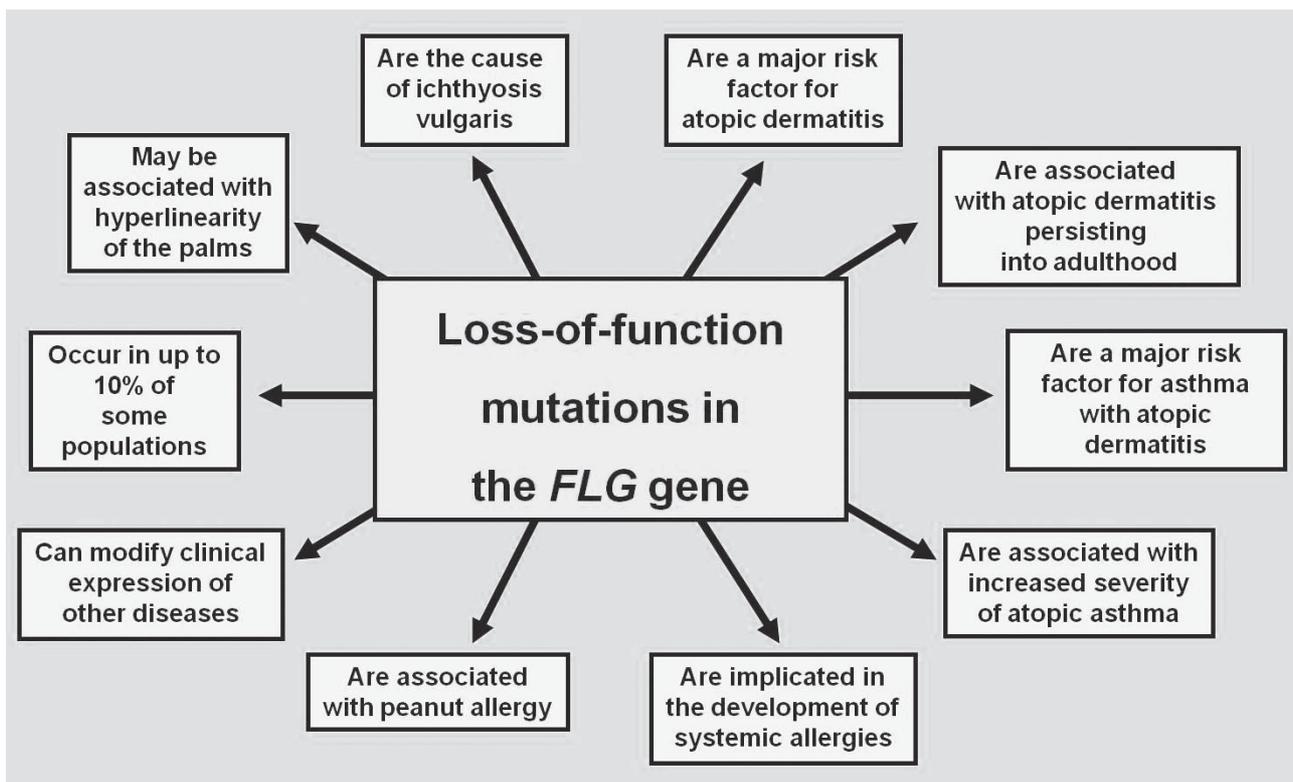

Figure 3. Clinical associations with mutations in the FLG gene.

Filaggrin and skin inflammation

The significance of filaggrin in the development of atopic dermatitis has also been suggested in cases of atopic dermatitis lacking specific mutations in *FLG*. Skin inflammation in atopic dermatitis is associated typically with increased expression of cytokines, such as interleukin-4 and -13, both of which have been shown to reduce *FLG* gene and protein expression in keratinocytes.[22] The cytokine interleukin-22 has also been shown to reduce *FLG* expression.[23] Moreover, polymorphisms in the genes encoding interleukin-10 and -13 can also impact on *FLG* expression.[24] In psoriasis, although there is no primary *FLG* gene association, treatment of patients with tumour necrosis factor-a antagonists has been shown to increase *FLG* expression in psoriatic skin and to improve the skin barrier.[25] Reduced FLG levels, either from a primary gene mutation or secondary reduction in protein expression, can also influence innate immune responses via toll-like receptor stimuli and thus contribute to skin inflammation.[26] *FLG* mutations have also been shown to affect the lipid composition of the skin barrier, with changes in the percentage of cholesterol, ceramide/cholesterol ratio, free fatty acids and triglycerides.[27]

Filaggrin therapeutics

The identification of reduced FLG expression in a range of dry, scaly disorders indicates that restoring FLG expression to improve skin barrier function could be a useful therapeutic endeavour.[28] How best to do this may depend on the underlying FLG abnormality. For example, one approach might be to try to upregulate expression of the *FLG* gene. To do this, pharmaceutical screening of small molecule/drug libraries to identify compounds capable of increasing *FLG* expression might generate new preparations suitable for the topical treatment of ichthyosis vulgaris and a subset of individuals with atopic dermatitis. There are also a number of currently available compounds that have been shown to increase FLG expression in keratinocytes. For example, oleanolic acid and ursolic acid, pentacyclic triterpenoids that naturally occur in many medicinal herbs and plants, have both been shown to improve skin barrier function by increasing FLG expression.[29] Alternatively, some drugs are currently being examined that can increase FLG in a different manner – by increasing read-through of a *FLG* gene mutation and thereby increase *FLG* messenger RNA and FLG protein levels. This approach is suitable for nonsense mutations in the *FLG* gene.[30] Compounds such as gentamicin can have this property in being able to increase FLG expression in skin, although alternative less toxic preparations are currently being investigated. The unifying goal is to try to develop topically applied FLG-promoting compounds that could surpass current therapies based on moisturizers, corticosteroids and other anti-inflammatory compounds.

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