One remarkable molecule: Filaggrin

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Abstract

The discovery, in 2006, that loss-of-function mutations in the filaggrin gene (*FLG*) are the cause of ichthyosis vulgaris – the most common disorder of keratinization – and also a strong genetic risk factor for atopic eczema, marked a significant breakthrough in the understanding of eczema pathogenesis. Subsequent investigations of the role of *FLG* null mutations have identified a series of significant associations with atopic disease phenotypes, including atopic asthma, allergic rhinitis and peanut allergy. However, many questions remain to be answered in relation to the precise mechanisms by which deficiency of an intracellular protein expressed primarily in the differentiating epidermis may contribute to the development of cutaneous and systemic pathology. This review aims to highlight the key milestones in filaggrin research over the past 25 years, to discuss the mechanistic, clinical and therapeutic implications and to consider possible future directions for ongoing investigation.

Keywords

Eczema; ichthyosis vulgaris; keratinizing disorder; atopy; skin barrier

A brief history of filaggrin, from ichthyosis vulgaris to modern molecular genetics

Ichthyosis vulgaris (OMIM #146700) is the most common inherited disorder of keratinisation, with an estimated prevalence of between 1 in 80 and 1 in 250 English schoolchildren (Brown *et al.*, 2008a; Wells and Kerr, 1966). It is an autosomal semidominant condition with incomplete penetrance and variable expressivity, such that disease severity can vary considerably even within affected families (Smith *et al.*, 2006). The ichthyosis is often asymptomatic, although patients may complain of dry, rough skin and cosmetic embarrassment. The disease is characterized by fine scaling which is most apparent on the lower abdomen, extensor aspects of arms and lower legs, with sparing of the flexures (Judge *et al.*, 2004). The semi-adherent scales are white or grey and the ichthyosis varies with environmental conditions, being more severe in cold, dry weather and sometimes resolving completely in a hot, humid environment (DiGiovanni, 2003). The palms and soles are not scaly, but show hyperlinearity, particularly on the thenar eminence (Brown *et al.*, 2009; Judge *et al.*, 2004). Keratosis pilaris is another feature of ichthyosis vulgaris (DiGiovanni, 2003; Mevorah *et al.*, 1985), but since keratosis pilaris is also very common in the normal population, it is not a discriminatory clinical sign (Brown *et al.*, 2008a).

Historically, several convergent lines of reasoning led to a study of the filaggrin gene as a cause for ichthyosis vulgaris. Skin biopsies from patients with ichthyosis vulgaris showed a reduction or absence of keratohyalin granules (the main component of which is profilaggrin) by light microscopy (Manabe *et al.*, 1991; Smith *et al.*, 2006; Sybert *et al.*, 1985) and by electron microscopy the granules were noted to be abnormally shaped or completely absent (Smith *et al.*, 2006; Sybert *et al.*, 1985). Immunostaining of ichthyosis vulgaris skin biopsies showed a reduction in filaggrin protein expression (Fleckman *et al.*, 1987; Sybert *et al.*, 1985) and a reduction in profilaggrin mRNA within keratinocytes was also reported (Manabe *et al.*, 1991; Nirunsuksiri *et al.*, 1995; Nirunsuksiri *et al.*, 1998). The recessive mutant flaky tail mouse, an animal model of ichthyosis vulgaris, demonstrated genetic linkage to the mouse epidermal differentiation complex (Smith *et al.*, 2006) and it expressed abnormal profilaggrin that is not proteolytically processed to filaggrin (Presland *et al.*, 2000). More recently, the phenotype of an absence of granules within the cells of the granular layer was mapped to the epidermal differentiation complex (Mischke *et al.*, 1996) on chromosome 1q21 (Compton *et al.*, 2002).

These observations date from the mid-1980s (Figure 1) and the profilaggrin gene was partially sequenced in 1992 (Presland *et al.*, 1992). However the identification of loss-of-function mutations was delayed until 2006 because *FLG* is such a very large and repetitive gene (Figure 2), making sequencing using conventional polymerase chain reaction (PCR) technically difficult. The formidable challenge of fully sequencing *FLG* exon 3 was eventually conquered by the use of long range PCR to amplify the whole of exon 3, combined with short specific PCRs to amplify multiple overlapping fragments which could then be used to reconstruct the repetitive sequence as a jig-saw (Figure 2)(Smith *et al.*, 2006). The initial two loss-of-function mutations (R501X and 2282del4) were thus identified in the first repeat of *FLG* exon 3 (Smith *et al.*, 2006).

The large and repetitive structure of the *FLG* gene remains a significant challenge for routine PCR-based sequencing. However, detailed knowledge of selected SNPs, which are unique to each repeat in the extensively studied white European DNA collections, has facilitated the development of repeat-selective PCR primers for sequencing and genotyping assays (Sandilands *et al.*, 2007). The use of next generation sequencing techniques for *FLG* genotyping is anticipated, but careful application will be required since it will be difficult to accurately align relatively short sequence reads (*e.g.* 50-100 base pairs) across the highly repetitive third exon, particularly in ethnic populations for which the *FLG* sequence is not yet well annotated.

The presence of intragenic copy number variation (CNV) in *FLG* adds a further level of complexity to the sequence analysis. Using Southern blot analysis, three common size alleles encoding 10, 11 or 12 filaggrin repeats were correctly inferred (Gan *et al.*, 1990). These were subsequently confirmed by sequencing and shown to be due to duplication of filaggrin repeat 8 or, duplication of both repeat 8 and repeat 10 (Sandilands *et al.*, 2007). Thus, null mutations aside, the number of filaggrin units varies from 20 to 24 in the human population. Very recently, methods have been developed to allow the genotyping of filaggrin CNV across large populations (Brown *et al.*, 2011b). The CNV allele frequencies in the Irish population were found to be 33.9% 10 repeats, 51.5% 11 repeats and 14.6% 12 repeats. The shortest genotype (10,10), carried by 11.5% of Irish people, was found to infer an eczema risk of 1.67, independent of the loss-of-function mutations. Interestingly, when null mutations are excluded, each additional filaggrin repeat gained decreases the odds ratio for atopic eczema by 0.88 (Brown *et al.*, 2011b). Thus, filaggrin CNV makes a significant, dose-dependent contribution to eczema risk and even a modest 10-20% increase in epidermal filaggrin expression is predicted to be therapeutic for eczema or protective against developing eczema.

It may be pertinent to ask why the strong association of *FLG* with atopic eczema was not detected by earlier genome-wide association studies? There are a number of possible explanations for this. One early genome-wide genetic study using microsatellite markers did show linkage to the 1q21 locus (Cookson *et al.*, 2001). Factors such as the type of markers used and the study design based on allele transmission (rather than the current more powerful case-control design using high density single-nucleotide polymorphisms – SNPs) led to the importance of this locus being under-recognized. Furthermore, the very strong association of *FLG* with atopic eczema that we now recognize, results from the combined null genotype reflecting the effect of two or more null mutations, a mechanism that genome-wide association using tagging SNPs is not designed to detect. The most recently published genome-wide study was designed with SNPs tagging the *FLG* locus and a strong signal was observed (Esparza-Gordillo *et al.*, 2009). It remains to be determined whether this signal is entirely attributable to *FLG* loss of function mutations or whether other genetic factors nearby in the epidermal differentiation complex of genes also contribute to eczema risk. CNV within the *FLG* gene contributes to eczema risk, independent of the null mutations (Brown et al., 2011b). This has yet to be factored into interpretation of the genome-wide association data for the 1q21 locus.

Profilaggrin and filaggrin are multi-functional proteins in the maintenance of an optimal skin barrier

The large (>400kDa), insoluble polyprotein profilaggrin is dephosphorylated and degraded to produce monomeric filaggrin in the stratum corneum and then further proteolyzed to release its component amino acids. Profilaggrin, filaggrin and the amino acids each make different contributions to epidermal structure and barrier function (Figure 3), but the exact mechanisms by which profilaggrin and filaggrin, as intracellular proteins, contribute to what appears to be a paracellular barrier defect (Gruber *et al.*, 2011; Scharschmidt *et al.*, 2009) remain to be defined.

Profilaggrin forms the major component of the keratohyalin granules that are visible by light microscopy within the granular layer of the epidermis. The profilaggrin molecule (Figure 2) is composed of an N-terminal domain which has calcium-binding and nuclear localization components, followed by 10, 11 or 12 nearly identical filaggrin repeats which have keratin-binding properties, plus a C-terminal domain of unknown function (Sandilands *et al.*, 2009). Each of these components has a different function in the differentiating epidermis. The S100-like calcium binding domain (Markova *et al.*, 1993) may play a role in the regulation of calcium-dependent events during terminal epidermal differentiation (Nirunsuksiri *et al.*, 1995; Presland *et al.*, 1992) or conversely, calcium may be involved in the control of profilaggrin processing (Presland *et al.*, 2004; Sandilands *et al.*, 2009). The N-terminal domain is cleaved from profilaggrin and translocates to the nucleus where it may play a role in the enucleation of keratinocytes in the outer stratum corneum (Sandilands *et al.*, 2009). The precise function of the C-terminal domain is unclear but it is known to be required for the processing of profilaggrin to filaggrin. Loss-of-function mutations within *FLG* exon 3 result in a truncated profilaggrin molecule which lacks the C-terminus, resulting in an almost complete absence of filaggrin monomers (Sandilands *et al.*, 2007). Each of the reported *FLG* null mutations therefore have an equivalent molecular biological effect, since they each produce biochemically unstable truncated profilaggrin which cannot be processed to release functional filaggrin.

Monomeric filaggrin binds to keratins 1 and 10 and other intermediate filament proteins within the keratinocyte cytoskeleton to form tight bundles (Manabe *et al.*, 1991), facilitating the collapse and flattening of cells in the outermost stratum corneum to produce squames. This process of cell flattening is still seen to occur in the absence of processed filaggrin, but

on an ultrastructural level, it appears that loss-of-function mutations in *FLG* may be associated with disorganized keratin filaments, impaired lamellar body loading and abnormal architecture of the lamellar bilayer (Gruber *et al.*, 2011). A reduction in corneodesmosome density and tight junction protein expression has also been observed in the skin from ichthyosis vulgaris patients (Gruber *et al.*, 2011). It is unclear as to how these observations may be attributable to filaggrin deficiency from our current understanding of filaggrin processing and they may represent secondary effects. Filaggrin contributes to the protein-lipid cornified cell envelope which replaces the plasma membrane of differentiating keratinocytes and is extensively cross-linked by transglutaminases, forming a barrier to water loss and minimizing the entry of allergens and micro-organisms (Candi *et al.*, 2005).

Filaggrin is ultimately deiminated and degraded on the skin surface to release its component amino acids. A mixture of hygroscopic amino acids, at an estimated concentration of 100 millimolar (Candi *et al.*, 2005) is produced, the so-called 'natural moisturising factor' (Rawlings and Harding, 2004), which contributes to epidermal hydration and barrier function. *FLG* null mutations are associated with lower levels of hygroscopic amino acids in the stratum corneum of human subjects, measured using Raman spectroscopy and there is a concomitant increase in transepidermal water loss (Kezic *et al.*, 2008).

Three published studies describing murine models of filaggrin haploinsufficiency have shown a barrier impairment and enhanced percutaneous allergen sensitization (Fallon *et al.*, 2009; Man *et al.*, 2008; Oyoshi *et al.*, 2009). The barrier integrity phenotype associated with *FLG* null mutations in human skin is currently emerging, with evidence of a significant reduction in natural moisturizing factors in the stratum corneum, to which filaggrin breakdown products contribute (Kezic *et al.*, 2008; Kezic *et al.*, 2011; O'Regan *et al.*, 2010b) as well as *FLG* genotype-related impairment in stratum corneum integrity and cohesion (Angelova-Fischer *et al.*, 2011). However, eczema severity is itself associated with a reduction in natural moisturizing factor concentrations and the debate is set to continue regarding the relative importance of epidermal defects and immune dysregulation as the key initiating factors in eczema pathogenesis (Elias and Schmuth, 2009; Hudson, 2006; Simpson, 2010; Vercelli, 2009).

Filaggrin is a histidine-rich protein (Lynley and Dale, 1983) and histidine is metabolized to trans-urocanic acid (trans-UCA); pyrrolidone-5-carboxylic acid (PCA) is the other main breakdown product of filaggrin and together these organic acids help to maintain the pH gradient of the epidermis as evidenced by a higher surface pH in *FLG* null mutation carriers (Jungersted *et al.*, 2010). The 'acid mantle' of the stratum corneum has a well-known antimicrobial effect and there is evidence that filaggrin breakdown products at physiological concentrations demonstrate an inhibitory effect on the growth of *Staphyloccus aureus* (Miajlovic *et al.*, 2010). An acidic pH within the stratum corneum is also important for the functional activity of enzymes involved in ceramide metabolism (Fluhr *et al.*, 2010). The acidic pH also modulates the activity of the serine protease cascade (Ovaere *et al.*, 2009) required for co-ordinated epidermal differentiation and cornified cell envelope formation. Finally, a possible role of filaggrin breakdown products in UV photo-protection has been suggested. The photo-isomerisation of trans- to cis-urocanic acid (Elias and Choi, 2005) produces a molecule with an action spectrum of 280-310nm (McLoone *et al.*, 2005) which is within the UVB range and there is *in vitro* evidence that cis-UCA has immunomodulatory actions in human keratinocytes and leukocytes (Gilmour *et al.*, 1993; McLoone *et al.*, 2005). There is also recent *in vitro* evidence from an organotypic siRNA *FLG* knock-down model that lack of filaggrin leads to a reduction in urocanic acid concentration and an increased sensitivity to UV-induced apoptosis (Mildner *et al.*, 2010).

Filaggrin and filaggrin-related proteins are expressed in the skin of mammals including monotremes, but these molecules are not well conserved and are difficult to identify in lower animal orders. Filaggrin biology has been most extensively studied in mouse and canine species (Chervet *et al.*; Fallon *et al.*, 2009; Hoste *et al.*; Man *et al.*, 2008; Marsella and Girolomoni, 2009; Matsui *et al.*; Moniaga *et al.*; Oyoshi *et al.*, 2009). These and other experimental models will continue to contribute to our understanding of the functions of this remarkable molecule.

Filaggrin haploinsufficiency plays a key role in phenotypic variation and common disorders of the skin

Filaggrin haploinsufficiency (that is, a lack of filaggrin protein resulting from a heterozyous loss-of-function mutation) contributes to several common dermatological disorders. Ichthyosis vulgaris is a semidominant disorder in which individuals with two *FLG* null mutations have a more severe phenotype than those with one *FLG* null mutation. The characteristic skin scaling in ichthyosis vulgaris may logically be attributed to the known functions of *FLG*, but the mechanisms by which *FLG* haploinsufficiency contributes to the associated features of keratosis pilaris and palmoplantar hyperlinearity are currently unknown.

Ichthyosis vulgaris is frequently associated with atopic eczema and hence it was a logical next step to investigate the association of *FLG* null mutations with this disease. A significant association was initially demonstrated in 15 Irish families and then replicated in three separate case/control studies from Irish, Scottish and Danish populations (Palmer *et al.*, 2006). The strong and highly significant association of *FLG* null mutations with atopic eczema has subsequently been replicated in over 20 independent studies, including case/control studies, family studies and unselected population cohorts. Two recent meta-analyses of these data have estimated the odds ratio of developing atopic eczema to be 4.78 (van den Oord and Sheikh, 2009) and 3.12 (Rodriguez *et al.*, 2009) in association with *FLG* null genotype. The eczema sub-phenotype that is most strongly associated with *FLG* null mutations is that of early onset, severe and persistent disease (Barker *et al.*, 2007; Brown *et al.*, 2008b) and with the associated 'extrinsic' features of raised total IgE and allergic sensitization (Weidinger *et al.*, 2006; Weidinger *et al.*, 2007). Population cohort studies include atopic eczema of mild-moderate severity and this phenotype has also demonstrated significant association with *FLG* null mutations (Brown *et al.*, 2008a; Gruber *et al.*, 2010; Henderson *et al.*, 2008). However, mild-moderate eczema shows a lower odds ratio than moderate-severe eczema and its association with *FLG* null mutations may be significant only in individuals carrying two null mutations (Brown *et al.*, 2008a). Taking into account all the clinical signs that may be attributable to *FLG* haploinsufficiency (including atopic eczema, ichthyosis, xerosis, palmar hyperlinearity and keratosis pilaris), *FLG* null mutations are highly penetrant (Brown *et al.*, 2009).

In the light of the very significant association of atopic eczema with *FLG* null mutations, several other sub-groups of eczema have been investigated for their link with filaggrin. There is a well recognized genetic susceptibility to contact dermatitis (Kezic, 2011) but it is often difficult to distinguish the sub-groups of irritant and allergic contact dermatitis from atopic dermatitis, hence these genetic studies are difficult to interpret (Brown and Cordell, 2008; de Jongh *et al.*, 2008; Lerbaek *et al.*, 2007). Studies on *FLG* genotype as a risk factor for experimentally-induced irritant dermatitis have given conflicting results (Angelova-Fischer *et al.*, 2011; Jungersted *et al.*, 2010) and this may be due to methodological differences in the concentration of irritant. Studies published to date have reported no association of *FLG* genotype with allergic contact dermatitis defined by a positive patch test result to one or more substance (Carlsen *et al.*, 2010) nor with a subset of patients having

both atopic eczema and contact allergy (Carlsen *et al.*, 2011b). In contrast, *FLG* null genotype has shown significant association with nickel sensitization, but only in a subgroup of cases reporting intolerance of costume jewellery (Novak *et al.*, 2008) and in a subgroup of women who had not had their ears pierced (Thyssen *et al.*, 2010b). The proposed explanation for this observation is that ear piercing is a stronger risk factor than *FLG* genotype (Ross-Hansen *et al.*, 2010), emphasising the importance of environmental exposures in allergy development as well as the need for careful phenotype definition in genetic studies.

One small study has investigated *FLG* genotype as a risk factor for a type I hypersensitivity reaction to latex (Carlsen *et al.*, 2011a) and found no significant association. However this study should be interpreted with caution since it included only three *FLG* heterozygotes and therefore may not have had sufficient power to exclude a significant association.

Another subgroup of atopic eczema is the severe viral infection eczema herpeticum. Case-control studies from European and African population groups have shown that the loss-of-function mutation R501X confers an even greater risk of eczema herpeticum than atopic eczema (Gao *et al.*, 2009), suggesting a possible role for defective skin barrier in this viral infection.

Two other common cutaneous disorders have been investigated with respect to a hypothetical *FLG* association: psoriasis, because of its co-association with the 1q21 locus in genome-wide scans (Bowcock *et al.*, 2001); and acne vulgaris, because of a possible protective effect of *FLG* null mutations (Sergeant *et al.*, 2009). However, adequately powered studies have shown that there is no association of *FLG* loss-of-function mutations with psoriasis in the UK and Irish populations (Zhao *et al.*, 2007) nor with acne vulgaris in the Singaporean Chinese population (Common *et al.*).

FLG mutations contribute to genetic risk at each step of the atopic march

The 'atopic march' (Hahn and Bacharier, 2005; Spergel, 2010; Wuthrich and Schmid-Grendelmeier, 2003) describes the tendency for atopic eczema to precede the development of food allergies, asthma and allergic rhinitis in a temporal sequence. *FLG* null mutations have been reported as a risk factor for each step in this march: atopic eczema (Palmer *et al.*, 2006); allergic sensitisation (van den Oord and Sheikh, 2009); the sub-group of asthma in association with eczema (Brown *et al.*, 2008a; Henderson *et al.*, 2008; Marenholz *et al.*, 2006; Weidinger *et al.*, 2008b); allergic rhinitis (Schuttelaar *et al.*, 2009; Weidinger *et al.*, 2008b); and most recently peanut allergy (Brown *et al.*, 2011). Thus, *FLG* null alleles are a significant risk factor for all aspects of atopy, but with differing odds ratios for each specific phenotype. Overall these studies underscore the role of skin barrier dysfunction as a key driver of allergic disease.

FLG loss-of-function mutations show population specificity

Since the discovery of the first two *FLG* loss-of-function mutations (R501X and 2282del4) in 2006 (Smith *et al.*, 2006), these two mutations along with the less prevalent S3247X and R2447X have been extensively studied and are present in 7-10% of the white European population. Subsequently a total of more than 20 other rare or family-specific loss-of-function mutations within *FLG* exon 3 have been discovered in populations of white European ancestry. However, these mutations are population-specific and cannot be used for the basis of genetic epidemiology studies in other populations. The population specificity of *FLG* null mutations indicates that they have arisen after one population has become split away from another. Thus populations arising from common ancestors share common ancestral mutations, but populations that have not experienced genetic admixture show

different mutations. The presence of rare and family-specific mutations indicates that *FLG* null mutations are still arising within the UK and Irish populations that we have studied.

Asian populations have been shown to have their own mutation spectra, following detailed study of the Japanese population (Enomoto *et al.*, 2008; Hamada *et al.*, 2008; Nemoto-Hasebe *et al.*, 2009b; Nomura *et al.*, 2008; Nomura *et al.*, 2009; Nomura *et al.*, 2007; Osawa *et al.*, 2010) and more recently the Han Chinese (Zhang *et al.*, 2011) and Singaporean Chinese populations (Chen *et al.*, 2011; Chen *et al.*, 2008). In the white European population two prevalent *FLG* mutations account for over 80% of the *FLG* null alleles, whereas in the Singaporean Chinese population there are eight different *FLG* null mutations that account for 80% of the spectrum of *FLG* mutations (Chen *et al.*, 2011).

The filaggrin story represents an unusual situation in complex trait genetics because the causative variants are either nonsense or frame-shift mutations in a protein-encoding exon and therefore new causative variants can be identified purely from their sequence. In contrast, the vast majority of loci detected in other complex traits are anonymous regions of DNA conferring disease susceptibility. Such loci are not clearly associated with a specific gene and the causative variant(s) either remain unknown or require considerable functional analysis to confirm and understand them. In the case of filaggrin, the readily identifiable causative mutations have revealed the complex genetic architecture of this locus, where each ancestral population has it own unique spectrum of mutations, some rare and some common, each of which are found on a different haplotype of nearby SNPs. Thus, individual SNPs can only reveal part of the signal coming from the *FLG* locus, further masking its importance in conferring eczema susceptibility.

A smaller number of *FLG* loss-of-function mutations have so far been reported in other Asian populations, including Korean (Kang *et al.*, 2009), Taiwanese (Hsu *et al.*, 2009), and Bangladeshi (Sinclair *et al.*, 2009) ichthyosis vulgaris and atopic eczema case collections plus one European patient whose mother was from the Philippines (Greisenegger *et al.*, 2010). Until recently, *FLG* null mutations had not been detected within African populations and an Ethiopian study, including direct sequencing of *FLG* in 40 cases, has identified only one loss-of-function mutation (Winge *et al.*). Furthermore, skin biopsies taken from 7 of the Ethiopian cases with palmar hyperlinearity did not show a reduction in filaggrin expression as expected on immunohistochemistry (Winge *et al.*). These are small numbers from which to draw any firm conclusions, but this study suggests that ichthyosis vulgaris in the Ethiopian population may have a different genetic mechanism from that seen in Europeans.

The prevalence of *FLG* null mutations varies across Europe, but R501X and 2282del4 are the two most common mutations and they have consistently shown significant association with atopic eczema across the continent (Rodriguez *et al.*, 2009), with the one exception of the Italian population. R501X and 2282del4 are rare in Italian atopic eczema cases (allele frequency <1% for each)(Giardina *et al.*, 2008) and full sequencing of *FLG* exon 3, exon 2 and the promoter region in a total of 220 Italian atopic dermatitis patients identified only 3 additional mutations and no association with atopic eczema (Cascella *et al.*, 2011). The pattern of *FLG* mutations in other Mediterranean populations has not yet been examined but the Italian data suggest that different genetic factors may predispose to atopic eczema in these populations and this warrants further investigation.

Filaggrin mutations may act as genetic modifiers in rare genodermatoses

It has been reported that the co-existence of *FLG* null mutations may be associated with a more severe phenotype of X-linked ichthyosis (Liao *et al.*, 2007) and pachyonychia congenita (Gruber *et al.*, 2009) within an affected family. However, it is not possible to be certain whether *FLG* is truly acting as a genetic modifier of disease in these single pedigrees

because each disease shows considerable inter-individual variation and greater numbers of cases will be needed to clarify this question.

What is the role of FLG-related barrier dysfunction in other inflammatory diseases?

Other inflammatory barrier diseases, including Crohn's disease, ulcerative colitis and sarcoidosis, share common susceptibility loci with atopic eczema (O'Regan *et al.*, 2010a; Schreiber *et al.*, 2005) but do not show association with *FLG* null mutations (Ruether *et al.*, 2006; Van Limbergen *et al.*, 2009).

Autoantibody formation to citrullinated profilaggrin is a highly sensitive and specific serological marker for rheumatoid arthritis (Sanmarti *et al.*, 2009) and can predate the onset of joint disease (Perez *et al.*, 2007). Levels of anti-filaggrin antibody may also correlate with arthritis activity (Choi *et al.*, 2005). However, filaggrin is not expressed in articular tissues and these autoantibodies appear to be produced as a result of cross-reaction between deiminated peptide sequences which are present within fibrin chains in the synovium (Mohrenschlager *et al.*, 2006) and deiminated sequences produced during the post-translational processing of profilaggrin (Perez *et al.*, 2007). Anti-filaggrin antibodies are therefore not believed to be pathogenic (Van Steendam *et al.*, 2011). *FLG* null mutations R501X, 2282del4 and 3702delG, have been studied in rheumatoid arthritis patients and controls (Huffmeier *et al.*, 2008) and *FLG* mutation carriers showed no increased risk of developing rheumatoid arthritis. However in a separate study *FLG* heterozygotes did show significantly elevated levels of autoantibodies to citrullinated peptides compared to controls (p=0.018)(Van Steendam *et al.*, 2011) and *FLG* null mutations may therefore contribute to the development of humoral autoimmunity in early rheumatoid arthritis (Huffmeier *et al.*, 2008) by an as yet undefined mechanism.

Alopecia areata is a tissue-specific autoimmune disease and genetic factors make a significant contribution to its aetiology (Petukhova *et al.*, 2010). Alopecia areata is known to be associated with atopic disease and it has been shown that co-morbidity with atopic eczema as well as the *FLG* null variants (R501X and 2282del4) predict a more severe form of alopecia areata (Betz *et al.*, 2007; Goh *et al.*, 2006).

FLG demonstrates gene-environment interactions

Eczema is a complex trait, in which multiple genetic and environmental factors contribute to pathogenesis and the interactions of several putative environmental risk factors for atopic disease with *FLG* genotype have been investigated. It has been hypothesized that *FLG* haploinsufficiency may weaken the epidermal barrier and hence potentiate the effects of environmental allergens (Bisgaard *et al.*, 2008) resulting in eczema. Birth cohort studies from Denmark and the UK have shown that cat ownership early in life increases the risk of atopic eczema as an additional effect on top of the risk associated with *FLG* null genotype, whereas dog exposure has no effect (Bisgaard *et al.*, 2008). A separate cohort study from the Netherlands has replicated this finding, showing an increased risk of atopic eczema in *FLG* null mutation carriers that is further enhanced by early-life exposure to cats (Schuttelaar *et al.*, 2009). However, these data are difficult to interpret mechanistically because, whilst other studies have shown an association of *FLG* null mutations with raised IgE to cat dander (Henderson *et al.*, 2008) and a significant correlation with atopic eczema severity and specific IgE level for cat dander (Nemoto-Hasebe *et al.*, 2009a), there was no such association with sensitization in the Danish or English cohorts.

Another key environmental influence early in life is the presence or absence of other children, including siblings and contacts in the day-care situation, since these individuals may result in contact with pathogens and allergens. Again these complex epidemiological

data are difficult to interpret. A risk analysis of early childhood eczema from a high-risk cohort of children born to mothers with asthma showed no association with time spent in day-care (Bisgaard *et al.*, 2009) but interaction with *FLG* genotype was not examined. Conversely, two large birth cohort studies from Germany (Cramer *et al.*, 2010) have shown that children with *FLG* null mutations have a significantly higher risk of eczema if they have an older sibling and attendance at a day-care centre lessened this risk (Cramer *et al.*, 2010). Environmental influences on eczema pathogenesis are therefore not clearly defined and these findings demonstrate the importance of stratification for *FLG* genotype in future epidemiological studies.

The development of irritant and allergic contact dermatitis is largely dependent on environmental exposure to the irritant or allergic substances, but *FLG* genotype is also a strong risk factor. However, as discussed above, it is currently unclear whether *FLG* haploinsufficiency is the risk *per se*, or whether the risk is mediated via atopic eczema.

The co-existence of *FLG* mutations and early food sensitization has been shown to improve the positive predictive value for childhood asthma (Marenholz *et al.*, 2009). This observation was interpreted as a representation of two distinct mechanisms interacting in the pathogenesis of asthma (Marenholz *et al.*, 2009). However, it is also possible that the increased risk of asthma occurs as a result of gene-environment interaction between *FLG* and food allergen exposure. A deeper understanding of the mechanisms within the atopic march will be required for the correct interpretation of these interesting observations.

Filaggrin deficiency forms one portion of a common pathway in eczema pathophysiology

The filaggrin story demonstrates how the study of a monogenic disorder can provide insight into complex trait disease and the striking significance of *FLG* null mutations as a genetic risk factor for atopic eczema has placed a new focus on the role of barrier impairment in eczema pathogenesis. There is an inherent barrier defect in atopic skin which is present even in the absence of eczema (Flohr *et al.*, 2010; Jakasa *et al.*, 2006; Jakasa *et al.*, 2007) and it is clear that barrier dysfunction can occur as a result of many different molecular mechanisms – independently of filaggrin – but these are outwith the scope of this current review. Focussing on filaggrin, it is apparent that not all filaggrin deficiency is related to *FLG* null mutations and down-regulation of filaggrin may be brought about by the interplay of other factors. The degree of barrier impairment assessed by transepidermal water loss and stratum corneum hydration shows some correlation with *FLG* null genotype (Jakasa *et al.*, 2011), although active inflammation further increases the degree of transepidermal water loss (Flohr *et al.*, 2010; Nemoto-Hasebe *et al.*, 2009a). Acute eczematous inflammation itself down-regulates filaggrin expression (Howell *et al.*, 2007) and inflammatory cytokines have been reported to down-regulate filaggrin expression *in vitro*, including interleukins-4 and −13 (Howell *et al.*, 2007), IL-22 (Gutowska-Owsiak *et al.*, 2011) and IL-25 (Hvid *et al.*, 2011b). Inflammatory cytokines may also down-regulate enzymes required for profilaggrin processing: IL-22 down-regulates cathepsin D *in vitro* (Gutowska-Owsiak *et al.*, 2011) and Th2-associated cytokines have been shown to reduce caspase 14 mRNA levels in cultured keratinocytes (Hvid *et al.*, 2011a). Similarly, genetic variation in enzymes known to play a role in profilaggrin processing, including caspase 14 (Denecker *et al.*, 2007; Hoste *et al.*, 2011), SASPase (Matsui *et al.*, 2011), calpain I and bleomycin hydrolase (Kamata *et al.*, 2011) may affect the levels of functional filaggrin monomers. In this way inflammatory mediators and enzymatic processing within the stratum corneum may each lead to filaggrin deficiency, forming a common pathway in eczema pathophysiology.

Large collaborative projects are required for the investigation of genetic effects on a population level

One of the hidden stories within the filaggrin field is the fact that many of the significant findings described above have resulted from the collaborative work of national and international groups of skin scientists and clinicians. The work required 'behind-the-scenes' to co-ordinate such collaborations is not formally reported, but is clearly an important part of the success of large studies. Similarly, the organization required to collect high quality samples and clinical data from birth cohorts, case collections and population-matched control groups is a very significant undertaking. Within the field of eczema research there are now several large cohorts and case collections which have been used to further our understanding of the role of *FLG* genotype, including the Irish case collection based in Dublin (Brown *et al.*, 2011; O'Regan *et al.*, 2010a; O'Regan *et al.*, 2010b; Sandilands *et al.*, 2007; Weidinger *et al.*, 2008a), MAS (Marenholz *et al.*, 2006), KORA (Novak *et al.*, 2008; Weidinger *et al.*, 2008a) and ETAC (Muller *et al.*, 2009) in Germany, GENUFAC in Northern Europe (Marenholz *et al.*, 2006), PIAMA in the Netherlands (Schuttelaar *et al.* 2009), COPSAC in Denmark (Bisgaard *et al.* 2008; Bisgaard *et al.* 2009), ALSPAC (Henderson *et al.*, 2008; Weidinger *et al.*, 2008a), NCCGP (Brown *et al.*, 2009; Brown *et al.*, 2008a) and the EAT Study (Flohr *et al.*, 2010) in England, ADVN in the USA (Gao *et al.*, 2009), a Danish population sample (Thyssen *et al.*, 2010a), a Canadian peanut allergy cohort (Brown *et al.*, 2011) and internationally, the ISAAC (Weidinger *et al.*, 2008b). These clinical resources will undoubtedly be useful in future studies and the established collaborative networks may be used to facilitate clinical trials forthcoming from the translational work as a result of new insight into genetic factors in eczema pathogenesis.

What may the next 25 years hold for the field of eczema genetic research?

Key strategic goals for the field of eczema genetics in the next 25 years may be categorized into understanding pathomechanisms, methods for the prevention of atopic disease and therapy development (Figure 1).

There are several rather fundamental questions that remain to be answered in relation to the filaggrin molecule: what are the patterns of filaggrin expression throughout the body; what are the mechanisms by which profilaggrin/filaggrin/amino acids act in healthy tissues; and how exactly does filaggrin deficiency lead to eczema and other atopic diseases? We can be reasonably optimistic that answers to these questions are likely to emerge over the coming years, particularly in view of the worldwide interest in filaggrin research. The field of complex trait genetics is progressing rapidly but one specific challenge is the dissection of the multiple gene-gene interactions that are likely to contribute to the heritability that remains to be explained in atopic eczema. Some genetic interactions with *FLG* have already been identified: *FLG* is known to show an independent and multiplicative effect with an eczema risk variant on 11q13.5 (O'Regan *et al.*, 2010a) and with polymorphisms in the genes encoding IL-10 and IL-13 (Lesiak *et al.*, 2011). However many other examples of genes interacting in the pathogenesis of atopic eczema are likely to emerge and these may well provide opportunities for disease prevention and novel therapies. It is hoped and anticipated that the application of next generation sequencing will speed up progress in the discovery of additional eczema genes. An understanding of the importance of *FLG* mutations in each population group will also facilitate the identification of other important genetic factors. In parallel with this, progress within the field of bioinformatics and statistical genetics is vital in order to make sense of the vast quantities of data produced by the latest high-throughput sequencing.

A clearer understanding of gene-environment interaction is needed in order to explain the dramatic rise in incidence of atopic disease in the industrialized nations over the recent decades. This will require careful epidemiological study of human populations, but the use of mouse models of atopic disease will also be useful in allowing experimental manipulation of both genetic and environmental effects. Gene-environment interactions are key to future preventative strategies, with the ultimate aim of preventing atopic eczema and halting the atopic march.

Another unanswered question is why filaggrin mutations are so prevalent in various human populations? It has been suggested that filaggrin deficiency may confer some heterozygote advantage and that "natural vaccination" against microbial pathogens via the skin barrier may have driven natural selection for these sequence variants during pandemics in our ancient past (Irvine & McLean, 2006). Filaggrin deficient animal models should allow this question to be addressed experimentally in the future (Fallon *et al.*, 2009).

There is potential for the application of filaggrin research to novel therapeutic approaches and personalized medicine

The understanding that has been gained from filaggrin research offers real potential for future improvements in the therapies available for atopic disease. It is clear that the clinical phenotype of ichthyosis vulgaris-related eczema has a poor prognosis in terms of atopic eczema as well as associated atopic asthma, allergic rhinitis and food allergy. We may therefore sub-classify atopic eczema cases according to *FLG* genotype for the purposes of pharmacogenetic studies. Feasibility studies are currently underway in the UK and USA to investigate the therapeutic potential of barrier enhancement using emollients (BEEP, 2011) and experimental evidence is emerging to show that the *FLG* gene is amenable to up-regulation. Clarification of the relative importance of the different functions of profilaggrin/filaggrin/amino acids (Figure 3) will allow the further development of strategies to enhance or replace these molecules within human skin.

It is noteworthy that eczema, in contrast to psoriasis, currently lacks any widely effective biological treatment and a clearer understanding of the key functional mechanisms in atopy will be required to identify appropriate biological targets. In the meantime, there is an opportunity to focus on barrier improvement with bespoke emollients such as ceramide-lipid (Elias, 2011) or filaggrin replacement. These barrier improvement therapies have a lower likelihood of undesirable systemic immune effects. Other potential therapeutic strategies include the use of a peroxisome-proliferator-activated receptor-alpha (PPARα) ligand along with topical corticosteroid therapy, since PPARα ligand has been shown in a mouse model to have anti-inflammatory synergy whilst reducing the barrier impairment that results from topical steroids used in isolation (Hatano *et al.*). TNF-alpha antagonists improve the skin barrier in psoriasis (Kim *et al.*, 2011) and therefore may also be beneficial in eczema.

Concluding remarks

In conclusion, the discovery of loss-of-function mutations within the filaggrin gene represents the single most significant breakthrough in understanding the molecular genetic mechanisms of a wide range of atopic and allergic disorders. Future research into the remarkable filaggrin molecule holds great potential to improve the care of atopic eczema patients and ultimately to prevent the development of atopic disease.

Acknowledgments

REFERENCES

Angelova-Fischer I, Mannheimer AC, Hinder A, et al. Distinct barrier integrity phenotypes in filaggrin-related atopic eczema following sequential tape stripping and lipid profiling. Exp Dermatol. 2011; 20:351–356. [PubMed: 21410766]

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; 127:564–567. [PubMed: 16990802]

BEEP. Feasibility Study of Barrier Enhancement for Eczema Prevention (BEEP). 2011. http://www.beepstudy.org/ and http://clinicaltrials.gov/ct2/show/NCT01142999

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; 127:2539–2543. [PubMed: 17581619]

Bisgaard H, Halkjaer LB, Hinge R, et al. Risk analysis of early childhood eczema. J Allergy Clin Immunol. 2009; 123:1355–1360. e1355. [PubMed: 19501236]

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:e131. [PubMed: 18578563]

Bowcock AM, Shannon W, Du F, et al. Insights into psoriasis and other inflammatory diseases from large-scale gene expression studies. Hum Mol Genet. 2001; 10:1793–1805. [PubMed: 11532989]

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. 2011a; 127:661–667. [PubMed: 21377035]

Brown SJ, Cordell HJ. Are filaggrin mutations associated with hand eczema or contact allergy?--we do not know. Br J Dermatol. 2008; 158:1383–1384. [PubMed: 18384470]

Brown SJ, Kroboth KE, Sandilands A, Campbell LE, Pohler E, Kezic S, et al. Intragenic copy number variation within filaggrin contributes to risk of atopic dermatitis with a dose-dependent effect. J Invest Dermatol. 2011b in press.

Brown SJ, Relton CL, Liao H, et al. Filaggrin haploinsufficiency is highly penetrant and is associated with increased severity of eczema: further delineation of the skin phenotype in a prospective epidemiological study of 792 school children. Br J Dermatol. 2009; 161:884–889. [PubMed: 19681860]

Brown SJ, Relton CL, Liao H, et al. Filaggrin null mutations and childhood atopic eczema: a population-based case-control study. J Allergy Clin Immunol. 2008a; 121:940–946. e943. [PubMed: 18313126]

Brown SJ, Sandilands A, Zhao Y, et al. Prevalent and low-frequency null mutations in the filaggrin gene are associated with early-onset and persistent atopic eczema. J Invest Dermatol. 2008b; 128:1591–1594. [PubMed: 18094728]

Candi E, Schmidt R, Melino G. The cornified envelope: a model of cell death in the skin. Nat Rev Mol Cell Biol. 2005; 6:328–340. [PubMed: 15803139]

Carlsen BC, Johansen JD, Menne T, et al. Filaggrin null mutations and association with contact allergy and allergic contact dermatitis: results from a tertiary dermatology clinic. Contact Dermatitis. 2010; 63:89–95. [PubMed: 20629673]

Carlsen BC, Meldgaard M, Hamann D, et al. Latex allergy and filaggrin null mutations. J Dent. 2011a; 39:128–132. [PubMed: 21074596]

Carlsen BC, Thyssen JP, Menne T, et al. Association between filaggrin null mutations and concomitant atopic dermatitis and contact allergy. Clin Exp Dermatol. 2011b; 36:467–472. [PubMed: 21434976]

Cascella R, Foti Cuzzola V, et al. Full sequencing of the FLG gene in Italian patients with atopic eczema: evidence of new mutations, but lack of an association. J Invest Dermatol. 2011; 131:982–984. [PubMed: 21289640]

Chen H, Common JE, Haines RL, et al. Wide spectrum of filaggrin-null mutations in atopic dermatitis highlights differences between Singaporean Chinese and European populations. Br J Dermatol. 2011

Chen H, Ho JC, Sandilands A, et al. Unique and recurrent mutations in the filaggrin gene in Singaporean Chinese patients with ichthyosis vulgaris. J Invest Dermatol. 2008; 128:1669–1675. [PubMed: 18239616]

Chervet L, Galichet A, McLean WH, et al. Missing C-terminal filaggrin expression, NFkappaB activation and hyperproliferation identify the dog as a putative model to study epidermal dysfunction in atopic dermatitis. Exp Dermatol. 19:e343–346. [PubMed: 20626465]

Choi KH, Lee EB, Yoo CD, et al. Clinical significance of anti-filaggrin antibody recognizing uncitrullinated filaggrin in rheumatoid arthritis. Exp Mol Med. 2005; 37:546–552. [PubMed: 16391515]

Common JE, Brown SJ, Haines RL, et al. Filaggrin null mutations are not a protective factor for acne vulgaris. J Invest Dermatol. 131:1378–1380. [PubMed: 21326297]

Compton JG, DiGiovanna JJ, Johnston KA, et al. Mapping of the associated phenotype of an absent granular layer in ichthyosis vulgaris to the epidermal differentiation complex on chromosome 1. Exp Dermatol. 2002; 11:518–526. [PubMed: 12473059]

Cookson WO, Ubhi B, Lawrence R, et al. Genetic linkage of childhood atopic dermatitis to psoriasis susceptibility loci. Nat Genet. 2001; 27:372–373. [PubMed: 11279517]

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; 125:1254–1260. e1255. [PubMed: 20513523]

de Jongh CM, Khrenova L, Verberk MM, et al. Loss-of-function polymorphisms in the filaggrin gene are associated with an increased susceptibility to chronic irritant contact dermatitis: a case-control study. Br J Dermatol. 2008; 159:621–627. [PubMed: 18637008]

Denecker G, Hoste E, Gilbert B, et al. Caspase-14 protects against epidermal UVB photodamage and water loss. Nat Cell Biol. 2007; 9:666–674. [PubMed: 17515931]

DiGiovanni, JJ. Ichthyosiform dermatoses. In: Freedberg, IM.; Eisen, AZ.; Wolff, K.; Austen, KF.; Goldsmith, LA.; Katz, SI., editors. Fitzpatrick's Dermatology in General Medicine. 6th ed.. Vol. 1. McGraw-Hill; 2003. p. 481-505.

Elias PM. Therapeutic Implications of a Barrier-based Pathogenesis of Atopic Dermatitis. Ann Dermatol. 2011; 22:245–254. [PubMed: 20711259]

Elias PM, Choi EH. Interactions among stratum corneum defensive functions. Exp Dermatol. 2005; 14:719–726. [PubMed: 16176279]

Elias PM, Schmuth M. Abnormal skin barrier in the etiopathogenesis of atopic dermatitis. Curr Opin Allergy Clin Immunol. 2009; 9:437–446. [PubMed: 19550302]

Enomoto H, Hirata K, Otsuka K, et al. Filaggrin null mutations are associated with atopic dermatitis and elevated levels of IgE in the Japanese population: a family and case-control study. J Hum Genet. 2008; 53:615–621. [PubMed: 18521703]

Esparza-Gordillo J, Weidinger S, Folster-Holst R, et al. A common variant on chromosome 11q13 is associated with atopic dermatitis. Nat Genet. 2009; 41:596–601. [PubMed: 19349984]

Fallon PG, Sasaki T, Sandilands A, et al. A homozygous frameshift mutation in the mouse Flg gene facilitates enhanced percutaneous allergen priming. Nat Genet. 2009; 41:602–608. [PubMed: 19349982]

Fleckman P, Holbrook KA, Dale BA, et al. Keratinocytes cultured from subjects with ichthyosis vulgaris are phenotypically abnormal. J Invest Dermatol. 1987; 88:640–645. [PubMed: 2437218]

Flohr C, England K, Radulovic S, et al. Filaggrin loss-of-function mutations are associated with early-onset eczema, eczema severity and transepidermal water loss at 3 months of age. Br J Dermatol. 2010; 163:1333–1336. [PubMed: 21137118]

Fluhr JW, Elias PM, Man MQ, et al. Is the filaggrin-histidine-urocanic acid pathway essential for stratum corneum acidification? J Invest Dermatol. 2010; 130:2141–2144. [PubMed: 20376063]

Gan SQ, McBride OW, Idler WW, Markova N, Steinert PM. Organization, structure, and polymorphisms of the human profilaggrin gene. Biochemistry. 1990; 29:9432–40. [PubMed: 2248957]

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; 124:507–513. 513 e501-507. [PubMed: 19733298]

Giardina E, Paolillo N, Sinibaldi C, et al. R501X and 2282del4 filaggrin mutations do not confer susceptibility to psoriasis and atopic dermatitis in Italian patients. Dermatology. 2008; 216:83–84. [PubMed: 18032906]

Gilmour JW, Vestey JP, George S, et al. Effect of phototherapy and urocanic acid isomers on natural killer cell function. J Invest Dermatol. 1993; 101:169–174. [PubMed: 8345217]

Goh C, Finkel M, Christos PJ, et al. Profile of 513 patients with alopecia areata: associations of disease subtypes with atopy, autoimmune disease and positive family history. J Eur Acad Dermatol Venereol. 2006; 20:1055–1060. [PubMed: 16987257]

Greisenegger E, Novak N, Maintz L, et al. Analysis of four prevalent filaggrin mutations (R501X, 2282del4, R2447X and S3247X) in Austrian and German patients with atopic dermatitis. J Eur Acad Dermatol Venereol. 2010; 24:607–610. [PubMed: 19874431]

Gruber R, Elias PM, Crumrine D, et al. Filaggrin genotype in ichthyosis vulgaris predicts abnormalities in epidermal structure and function. Am J Pathol. 2011; 178:2252–2263. [PubMed: 21514438]

Gruber R, Janecke AR, Grabher D, et al. Lower prevalence of common filaggrin mutations in a community sample of atopic eczema: is disease severity important? Wien Klin Wochenschr. 2010; 122:551–557. [PubMed: 20865458]

Gruber R, Wilson NJ, Smith FJ, et al. Increased pachyonychia congenita severity in patients with concurrent keratin and filaggrin mutations. Br J Dermatol. 2009; 161:1391–1395. [PubMed: 19785597]

Gutowska-Owsiak D, Schaupp AL, Salimi M, et al. IL-22 down-regulates filaggrin expression and affects expression of profilaggrin processing enzymes. Br J Dermatol. 2011

Hahn EL, Bacharier LB. The atopic march: the pattern of allergic disease development in childhood. Immunol Allergy Clin North Am. 2005; 25:231–246. v. [PubMed: 15878453]

Hamada T, Sandilands A, Fukuda S, et al. De novo occurrence of the filaggrin mutation p.R501X with prevalent mutation c.3321delA in a Japanese family with ichthyosis vulgaris complicated by atopic dermatitis. J Invest Dermatol. 2008; 128:1323–1325. [PubMed: 18007582]

Hatano Y, Elias PM, Crumrine D, et al. Efficacy of Combined Peroxisome Proliferator-Activated Receptor-alpha Ligand and Glucocorticoid Therapy in a Murine Model of Atopic Dermatitis. J Invest Dermatol.

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; 121:872–877. e879. [PubMed: 18325573]

Hoste E, Kemperman P, Devos M, et al. Caspase-14 Is Required for Filaggrin Degradation to Natural Moisturizing Factors in the Skin. J Invest Dermatol.

Hoste E, Kemperman P, Devos M, et al. Caspase-14 Is Required for Filaggrin Degradation to Natural Moisturizing Factors in the Skin. J Invest Dermatol. 2011

Howell MD, Kim BE, Gao P, et al. Cytokine modulation of atopic dermatitis filaggrin skin expression. J Allergy Clin Immunol. 2007; 120:150–155. [PubMed: 17512043]

Hsu CK, Akiyama M, Nemoto-Hasebe I, et al. Analysis of Taiwanese ichthyosis vulgaris families further demonstrates differences in FLG mutations between European and Asian populations. Br J Dermatol. 2009; 161:448–451. [PubMed: 19416262]

Hudson TJ. Skin barrier function and allergic risk. Nat Genet. 2006; 38:399–400. [PubMed: 16570058]

Huffmeier U, Boiers U, Lascorz J, et al. Loss-of-function mutations in the filaggrin gene: no contribution to disease susceptibility, but to autoantibody formation against citrullinated peptides in early rheumatoid arthritis. Ann Rheum Dis. 2008; 67:131–133. [PubMed: 17704064]

Hvid M, Johansen C, Deleuran B, et al. Regulation of caspase 14 expression in keratinocytes by inflammatory cytokines - a possible link between reduced skin barrier function and inflammation? Exp Dermatol. 2011a

Hvid M, Vestergaard C, Kemp K, et al. IL-25 in Atopic Dermatitis: A Possible Link between Inflammation and Skin Barrier Dysfunction? J Invest Dermatol. 2011b

Jakasa I, de Jongh CM, Verberk MM, et al. Percutaneous penetration of sodium lauryl sulphate is increased in uninvolved skin of patients with atopic dermatitis compared with control subjects. Br J Dermatol. 2006; 155:104–109. [PubMed: 16792760]

Jakasa I, Koster ES, Calkoen F, et al. Skin barrier function in healthy subjects and patients with atopic dermatitis in relation to filaggrin loss-of-function mutations. J Invest Dermatol. 2011; 131:540–542. [PubMed: 20962854]

Jakasa I, Verberk MM, Esposito M, et al. Altered penetration of polyethylene glycols into uninvolved skin of atopic dermatitis patients. J Invest Dermatol. 2007; 127:129–134. [PubMed: 17039242]

Judge, MR.; McLean, WHI.; Munro, CS. Disorders of keratinization. In: Burns, T.; Cox, N.; Griffiths, C., editors. Rook's Textbook of Dermatology. 7th ed.. Vol. 34. Blackwell Publishing; 2004. p. 37-34.10

Jungersted JM, Scheer H, Mempel M, et al. Stratum corneum lipids, skin barrier function and filaggrin mutations in patients with atopic eczema. Allergy. 2010; 65:911–918. [PubMed: 20132155]

Kamata Y, Yamamoto M, Kawakami F, et al. Bleomycin hydrolase is regulated biphasically in a differentiation- and cytokine-dependent manner: relevance to atopic dermatitis. J Biol Chem. 2011; 286:8204–8212. [PubMed: 21190945]

Kang TW, Lee JS, Oh SW, et al. Filaggrin mutation c.3321delA in a Korean patient with ichthyosis vulgaris and atopic dermatitis. Dermatology. 2009; 218:186–187. [PubMed: 18841000]

Kezic S. Genetic susceptibility to occupational contact dermatitis. Int J Immunopathol Pharmacol. 2011; 24:73S–78S. [PubMed: 21329569]

Kezic S, Kemperman PM, Koster ES, et al. Loss-of-function mutations in the filaggrin gene lead to reduced level of natural moisturizing factor in the stratum corneum. J Invest Dermatol. 2008; 128:2117–2119. [PubMed: 18305568]

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

Kim BE, Howell MD, Guttman E, et al. TNF-alpha downregulates filaggrin and loricrin through c-Jun N-terminal kinase: role for TNF-alpha antagonists to improve skin barrier. J Invest Dermatol. 2011; 131:1272–1279. [PubMed: 21346775]

Lerbaek A, Bisgaard H, Agner T, et al. Filaggrin null alleles are not associated with hand eczema or contact allergy. Br J Dermatol. 2007; 157:1199–1204. [PubMed: 17970802]

Lesiak A, Kuna P, Zakrzewski M, et al. Combined occurrence of filaggrin mutations and IL-10 or IL-13 polymorphisms predisposes to atopic dermatitis. Exp Dermatol. 2011; 20:491–495. [PubMed: 21426411]

Liao H, Waters AJ, Goudie DR, et al. Filaggrin mutations are genetic modifying factors exacerbating X-linked ichthyosis. J Invest Dermatol. 2007; 127:2795–2798. [PubMed: 17657246]

Lynley AM, Dale BA. The characterization of human epidermal filaggrin. A histidine-rich, keratin filament-aggregating protein. Biochim Biophys Acta. 1983; 744:28–35. [PubMed: 6187370]

Man MQ, Hatano Y, Lee SH, et al. Characterization of a hapten-induced, murine model with multiple features of atopic dermatitis: structural, immunologic and biochemical changes following single versus multiple oxazolone challenges. J Invest Dermatol. 2008; 128:79–86. [PubMed: 17671515]

Manabe M, Sanchez M, Sun TT, et al. Interaction of filaggrin with keratin filaments during advanced stages of normal human epidermal differentiation and in ichthyosis vulgaris. Differentiation. 1991; 48:43–50. [PubMed: 1720750]

Marenholz I, Kerscher T, Bauerfeind A, et al. An interaction between filaggrin mutations and early food sensitization improves the prediction of childhood asthma. J Allergy Clin Immunol. 2009; 123:911–916. [PubMed: 19348926]

Marenholz I, Nickel R, Ruschendorf F, et al. Filaggrin loss-of-function mutations predispose to phenotypes involved in the atopic march. J Allergy Clin Immunol. 2006; 118:866–871. [PubMed: 17030239]

Markova NG, Marekov LN, Chipev CC, et al. Profilaggrin is a major epidermal calcium-binding protein. Mol Cell Biol. 1993; 13:613–625. [PubMed: 8417356]

Marsella R, Girolomoni G. Canine models of atopic dermatitis: a useful tool with untapped potential. J Invest Dermatol. 2009; 129:2351–2357. [PubMed: 19516261]

Matsui T, Miyamoto K, Kubo A, et al. SASPase regulates stratum corneum hydration through profilaggrin-to-filaggrin processing. EMBO Mol Med. 2011; 3:320–333. [PubMed: 21542132]

McLoone P, Simics E, Barton A, et al. An action spectrum for the production of cis-urocanic acid in human skin in vivo. J Invest Dermatol. 2005; 124:1071–1074. [PubMed: 15854050]

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; 112:679–685. [PubMed: 4005167]

Miajlovic H, Fallon PP, Irvine AD, et al. Effect of filaggrin breakdown products on growth of and protein expression by Staphylococcus aureus. J Allergy Clin Immunol. 2010

Mildner M, Jin J, Eckhart L, et al. Knockdown of filaggrin impairs diffusion barrier function and increases UV sensitivity in a human skin model. J Invest Dermatol. 2010; 130:2286–2294. [PubMed: 20445547]

Mischke D, Korge BP, Marenholz I, et al. Genes encoding structural proteins of epidermal cornification and S100 calcium-binding proteins form a gene complex ("epidermal differentiation complex") on human chromosome 1q21. J Invest Dermatol. 1996; 106:989–992. [PubMed: 8618063]

Mohrenschlager M, Schafer T, Huss-Marp J, et al. The course of eczema in children aged 5-7 years and its relation to atopy: differences between boys and girls. Br J Dermatol. 2006; 154:505–513. [PubMed: 16445783]

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. 176:2385–2393. [PubMed: 20304960]

Muller S, Marenholz I, Lee YA, et al. Association of Filaggrin loss-of-function-mutations with atopic dermatitis and asthma in the Early Treatment of the Atopic Child (ETAC) population. Pediatr Allergy Immunol. 2009; 20:358–361. [PubMed: 19538357]

Nemoto-Hasebe I, Akiyama M, Nomura T, et al. Clinical severity correlates with impaired barrier in filaggrin-related eczema. J Invest Dermatol. 2009a; 129:682–689. [PubMed: 18818676]

Nemoto-Hasebe I, Akiyama M, Nomura T, et al. FLG mutation p.Lys4021X in the C-terminal imperfect filaggrin repeat in Japanese patients with atopic eczema. Br J Dermatol. 2009b; 161:1387–1390. [PubMed: 19663875]

Nirunsuksiri W, Presland RB, Brumbaugh SG, et al. Decreased profilaggrin expression in ichthyosis vulgaris is a result of selectively impaired posttranscriptional control. J Biol Chem. 1995; 270:871–876. [PubMed: 7822325]

Nirunsuksiri W, Zhang SH, Fleckman P. Reduced stability and bi-allelic, coequal expression of profilaggrin mRNA in keratinocytes cultured from subjects with ichthyosis vulgaris. J Invest Dermatol. 1998; 110:854–861. [PubMed: 9620289]

Nomura T, Akiyama M, Sandilands A, et al. Specific filaggrin mutations cause ichthyosis vulgaris and are significantly associated with atopic dermatitis in Japan. J Invest Dermatol. 2008; 128:1436–1441. [PubMed: 18200065]

Nomura T, Akiyama M, Sandilands A, et al. Prevalent and rare mutations in the gene encoding filaggrin in Japanese patients with ichthyosis vulgaris and atopic dermatitis. J Invest Dermatol. 2009; 129:1302–1305. [PubMed: 19037238]

Nomura T, Sandilands A, Akiyama M, et al. Unique mutations in the filaggrin gene in Japanese patients with ichthyosis vulgaris and atopic dermatitis. J Allergy Clin Immunol. 2007; 119:434–440. [PubMed: 17291859]

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; 128:1430–1435. [PubMed: 18049447]

O'Regan GM, Campbell LE, Cordell HJ, et al. Chromosome 11q13.5 variant associated with childhood eczema: an effect supplementary to filaggrin mutations. J Allergy Clin Immunol. 2010a; 125:170–174. e171–172.

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. 2010b; 126:574–580. e571.

Osawa R, Konno S, Akiyama M, et al. Japanese-specific filaggrin gene mutations in Japanese patients suffering from atopic eczema and asthma. J Invest Dermatol. 2010; 130:2834–2836. [PubMed: 20686498]

Ovaere P, Lippens S, Vandenabeele P, et al. The emerging roles of serine protease cascades in the epidermis. Trends Biochem Sci. 2009; 34:453–463. [PubMed: 19726197]

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; 124:485–493. 493 e481. [PubMed: 19665780]

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; 38:441–446. [PubMed: 16550169]

Perez ML, Gomara MJ, Ercilla G, et al. Antibodies to citrullinated human fibrinogen synthetic peptides in diagnosing rheumatoid arthritis. J Med Chem. 2007; 50:3573–3584. [PubMed: 17585853]

Petukhova L, Duvic M, Hordinsky M, et al. Genome-wide association study in alopecia areata implicates both innate and adaptive immunity. Nature. 2010; 466:113–117. [PubMed: 20596022]

Presland RB, Boggess D, Lewis SP, et al. 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; 115:1072–1081. [PubMed: 11121144]

Presland RB, Coulombe PA, Eckert RL, et al. Barrier function in transgenic mice overexpressing K16, involucrin, and filaggrin in the suprabasal epidermis. J Invest Dermatol. 2004; 123:603–606. [PubMed: 15304104]

Presland RB, Haydock PV, Fleckman P, et al. Characterization of the human epidermal profilaggrin gene. Genomic organization and identification of an S-100-like calcium binding domain at the amino terminus. J Biol Chem. 1992; 267:23772–23781. [PubMed: 1429717]

Rawlings AV, Harding CR. Moisturization and skin barrier function. Dermatol Ther. 2004; 17(Suppl 1):43–48. [PubMed: 14728698]

Rodriguez E, Baurecht H, Herberich E, et al. Meta-analysis of filaggrin polymorphisms in eczema and asthma: robust risk factors in atopic disease. J Allergy Clin Immunol. 2009; 123:1361–1370. e1367. [PubMed: 19501237]

Ross-Hansen K, Menne T, Johansen JD, et al. Nickel reactivity and filaggrin null mutations-- evaluation of the filaggrin bypass theory in a general population. Contact Dermatitis. 2010; 64:24–31. [PubMed: 21166815]

Ruether A, Stoll M, Schwarz T, et al. Filaggrin loss-of-function variant contributes to atopic dermatitis risk in the population of Northern Germany. Br J Dermatol. 2006; 155:1093–1094. [PubMed: 17034559]

Sandilands A, Sutherland C, Irvine A, et al. Filaggrin in the frontline: role in skin barrier function and disease. J Cell Sci. 2009; 122:1285–1294. [PubMed: 19386895]

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; 39:650–654. [PubMed: 17417636]

Sanmarti R, Graell E, Perez ML, et al. Diagnostic and prognostic value of antibodies against chimeric fibrin/filaggrin citrullinated synthetic peptides in rheumatoid arthritis. Arthritis Res Ther. 2009; 11:R135. [PubMed: 19725967]

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. 506 e491-496. [PubMed: 19733297]

Schreiber S, Rosenstiel P, Albrecht M, et al. Genetics of Crohn disease, an archetypal inflammatory barrier disease. Nat Rev Genet. 2005; 6:376–388. [PubMed: 15861209]

Schuttelaar ML, Kerkhof M, Jonkman MF, et al. Filaggrin mutations in the onset of eczema, sensitization, asthma, hay fever and the interaction with cat exposure. Allergy. 2009; 64:1758–1765. [PubMed: 19839980]

Sergeant A, Campbell LE, Hull PR, et al. Heterozygous null alleles in filaggrin contribute to clinical dry skin in young adults and the elderly. J Invest Dermatol. 2009; 129:1042–1045. [PubMed: 18987673]

Simpson E. Are epidermal defects the key initiating factors in the development of atopic dermatitis? Br J Dermatol. 2010; 163:1147–1148. [PubMed: 21137113]

Sinclair C, O'Toole EA, Paige D, et al. Filaggrin mutations are associated with ichthyosis vulgaris in the Bangladeshi population. Br J Dermatol. 2009; 160:1113–1115. [PubMed: 19239468]

Smith FJ, Irvine AD, Terron-Kwiatkowski A, et al. Loss-of-function mutations in the gene encoding filaggrin cause ichthyosis vulgaris. Nat Genet. 2006; 38:337–342. [PubMed: 16444271]

Spergel JM. From atopic dermatitis to asthma: the atopic march. Ann Allergy Asthma Immunol. 2010; 105:99–106. quiz 107-109, 117. [PubMed: 20674819]

Sybert VP, Dale BA, Holbrook KA. Ichthyosis vulgaris: identification of a defect in synthesis of filaggrin correlated with an absence of keratohyaline granules. J Invest Dermatol. 1985; 84:191–194. [PubMed: 2579164]

Thyssen JP, Carlsen BC, Menne T, et al. Filaggrin null mutations increase the risk and persistence of hand eczema in subjects with atopic dermatitis: results from a general population study. Br J Dermatol. 2010a; 163:115–120. [PubMed: 20426775]

Thyssen JP, Johansen JD, Linneberg A, et al. The association between null mutations in the filaggrin gene and contact sensitization to nickel and other chemicals in the general population. Br J Dermatol. 2010b

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. [PubMed: 19589816]

Van Limbergen J, Russell RK, Nimmo ER, et al. Filaggrin loss-of-function variants are associated with atopic comorbidity in pediatric inflammatory bowel disease. Inflamm Bowel Dis. 2009; 15:1492–1498. [PubMed: 19408338]

Van Steendam K, Tilleman K, Deforce D. The relevance of citrullinated vimentin in the production of antibodies against citrullinated proteins and the pathogenesis of rheumatoid arthritis. Rheumatology (Oxford). 2011; 50:830–837. [PubMed: 21278075]

Vercelli D. Of flaky tails and itchy skin. Nat Genet. 2009; 41:512–513. [PubMed: 19399034]

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. 2008a; 122:560–568. e564. [PubMed: 18774391]

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; 118:214–219. [PubMed: 16815158]

Weidinger S, O'Sullivan M, Illig T, et al. Filaggrin mutations, atopic eczema, hay fever, and asthma in children. J Allergy Clin Immunol. 2008b; 121:1203–1209. e1201. [PubMed: 18396323]

Weidinger S, Rodriguez E, Stahl C, et al. Filaggrin mutations strongly predispose to early-onset and extrinsic atopic dermatitis. J Invest Dermatol. 2007; 127:724–726. [PubMed: 17096018]

Wells RS, Kerr CB. Clinical features of autosomal dominant and sex-linked ichthyosis in an English population. Br Med J. 1966; 1:947–950. [PubMed: 20790920]

Winge MC, Bilcha KD, Lieden A, et al. Novel filaggrin mutation but no other loss-of-function variants found in ethiopian atopic-dermatitis patients. Br J Dermatol.

Wuthrich B, Schmid-Grendelmeier P. The atopic eczema/dermatitis syndrome. Epidemiology, natural course, and immunology of the IgE-associated ("extrinsic") and the nonallergic ("intrinsic") AEDS. J Investig Allergol Clin Immunol. 2003; 13:1–5.

Zhang H, Guo Y, Wang W, et al. Mutations in the filaggrin gene in Han Chinese patients with atopic dermatitis. Allergy. 2011
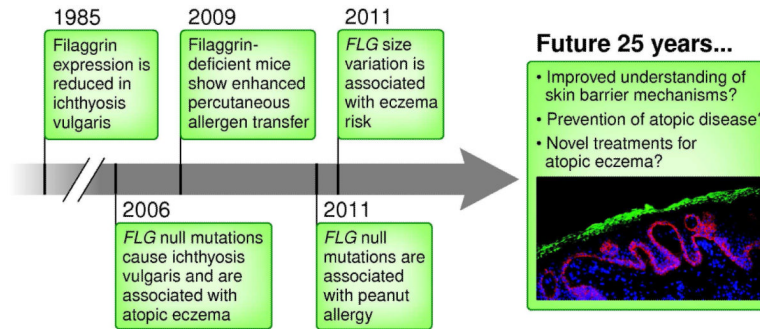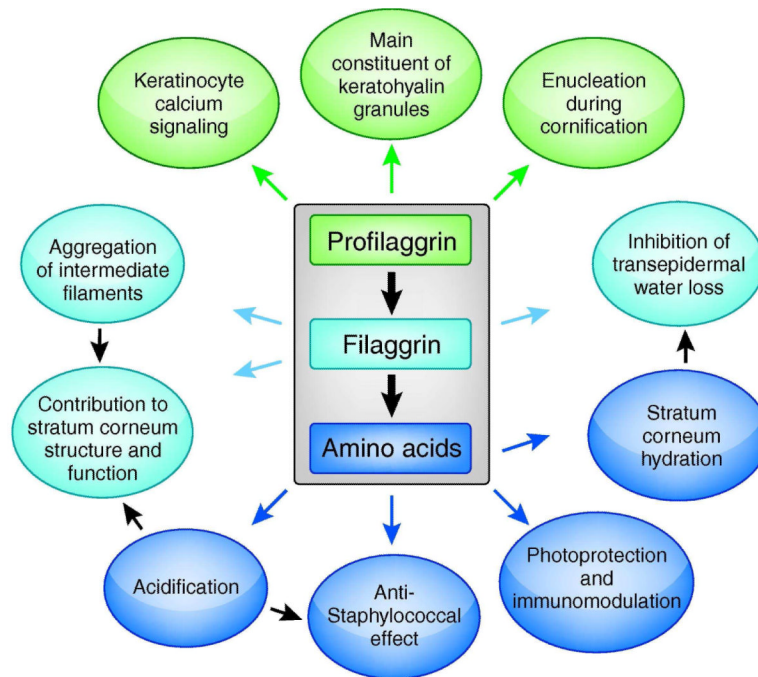
Figure 1.
Historical perspective on filaggrin research
Insert shows immunohistochemical staining of human epidermis, with filaggrin in green,
basal-specific keratin 5 in red and nuclei stained blue.
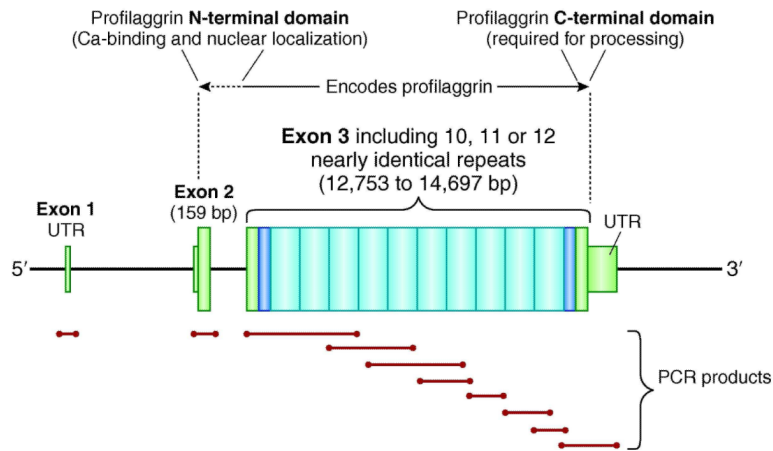
Figure 2.
Diagrammatic representation of *FLG* gene structure and sequencing strategy?
FLG is a large gene located on chromosome 1q21, within the epidermal differentiation complex, a dense cluster of genes involved with keratinocyte terminal differentiation. The gene product is profilaggrin, an insoluble polyprotein which is proteolyzed to release functional filaggrin monomers. Full sequencing of the *FLG* gene is time-consuming and requires 10 or more PCR reactions and more than 30 sequencing reactions. UTR, untranslated region; bp, base pairs; PCR, polymerase chain reaction

Figure 3.
Profilaggrin, filaggrin and their constituent amino acids are multifunctional proteins
contributing to the formation and function of the skin barrier
Diagram summarizing the known and possible functions of profilaggrin, filaggrin and amino
acids released by filaggrin proteolysis.