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# Ichthyosis Update: Towards a Function-Driven Model of Pathogenesis of the Disorders of Cornification and the Role of Corneocyte Proteins in These Disorders

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## Abstract

The genetic causes of most of the disorders of cornification have been uncovered. We now face the significant task of delineating how these mutations result in specific phenotypes. Because the permeability barrier resides in the extracellular lipid-enriched domains of the stratum corneum, it was anticipated that disorders of lipid metabolism would perturb the lamellar membrane structures of the extracellular domains and would result in a defective barrier. Unanticipated was the finding that inherited disorders of corneocyte proteins also exhibit, to varying degrees, an impaired permeability barrier. The effect of these corneocyte mutations on barrier function have shed light on how corneocytes interact with the intercellular lamellae to provide the barrier. In some entities, an impaired scaffold leads to fragmented and foreshortened lamellar membranes (e.g., transglutaminase-deficient lamellar ichthyosis, loricrin keratoderma). In others, there is impaired lamellar body secretion (e.g., epidermolytic hyperkeratosis) and altered lipid processing (e.g., Netherton syndrome), leading to deficiency of lamellar membrane structures. The combined insights from delineation of the pathogenesis of lipid metabolic defects and corneocyte protein abnormalities can be used to develop a function-driven model of disease pathogenesis. This model will aid in the development of more targeted approaches to therapy and in understanding some systemic complications of these disorders.

## Introduction

The ichthyoses comprise a large group of scaling disorders with diverse etiology [1–4]. To date, more than 25 genes have been identified that encode a wide spectrum of epidermal proteins, including enzymes of lipid metabolism and of peptide cross-linking, proteases and their inhibitors, epidermal structural proteins, and proteins involved in cellular communication, signaling and gene transcription. Abnormalities in any of these components result in a rather stereotypic epidermal response with epidermal hyperplasia and the formation of excess stratum corneum (SC) accompanied by abnormal (delayed and/or disordered) desquamation, with visible accumulation of squames (scales) on the skin's surface – the clinical hallmark of all the ichthyoses (Table 1). In certain instances, there is at least transiently increased skin fragility or blistering, as in Netherton syndrome and epidermolytic hyperkeratosis (EHK). In this

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review, we examine the role of corneocyte proteins in disorders of cornification using a new, function-driven model of pathogenesis. The role of lipid metabolic defects in these disorders is reviewed elsewhere [5].

## A Function-Driven Model of Disease Pathogenesis in Disorders of Cornification

As more has been learned about these disorders, various classification systems have been proposed. In the 1960's, Frost and Weinstein offered a classification based upon epidermal kinetics, in which disorders were designated either primarily retention hyperkeratoses (delayed desquamation with normal rates of epidermal renewal) or hyperproliferative states [6]. In the 1980's Elias and Williams advanced a morphological classification with disorders affecting the intercellular lipids ("mortar") and those affecting the structural proteins of the corneocyte ("bricks")[7, 8]. As further insights have been gained into the relationships between stratum corneum structures and the barrier functions of epidermis [9], it is now possible to integrate the epidermal kinetics model with the "Bricks and Mortar" model. This new, function-driven model provides a framework for understanding how such a wide and disparate group of genetic errors results in the ichthyosis phenotype. Of particular importance in the function-driven model is the observation that epidermal permeability barrier function is abnormal to a varying extent in virtually all of these disorders (Table 2).

The stratum corneum (SC) comprises a unique, two-compartment system of protein-enriched corneocytes, embedded in a lipid-enriched extracellular matrix. SC lipids are composed of extremely hydrophobic species, and organized into repeating arrays of lamellar membranes (Fig. 1). These membranes provide the permeability barrier, which can be demonstrated ultrastructurally using a small, electron-dense water-soluble tracer molecule, lanthanum that follows the outward movement of water in the epidermis. In normal epidermis, the movement of lanthanum is halted at the stratum granulosum – SC interface by the interposition of lamellar membrane structures in the intercellular domain of the SC [10, 11]. If the lamellar structures are inherently abnormal, as in many ichthyoses, or removed from the SC, as for example, by solvent extraction, transepidermal water loss (TEWL) rates increase, and lanthanum then can be seen to penetrate the SC through the extracellular pathway (Fig. 2). Acute experimental perturbations of the permeability barrier (e.g., through solvent extraction or tape strippings) result in a series of homeostatic responses aimed at repairing the barrier [12, 13]. In the first wave of responses, occurring within minutes of barrier disruption, the loss of the high calcium milieu bathing the stratum granulosum (SG), signals secretion of preformed lamellar bodies from the upper SG ("Deliver more lipid, now!") (Fig. 3). In the second phase, occurring within hours and signaled in part by release of preformed IL-1α from SC stores, epidermal lipid synthesis increases ("Make more lipid!"). In the third phase, within a day and also in response to cytokine signaling, epidermal DNA synthesis increases ("Make more cells!"). In normal human epidermis, these responses result in repair of the permeability barrier within about 3 days. In disorders of cornification, where a mutation produces a barrier defect that cannot be corrected by these homeostatic responses, the repair efforts (hypermetabolism, hyperplasia) do not terminate. Thus, a mutation that produces a barrier defect will invariably be associated with epidermal hyperplasia.

The corneocytes 'bricks' are constituents of the permeability barrier by two mechanisms. First, corneocytes serve as a critical *scaffold*, required for the organization of the extracellular lipid matrix into its characteristic lamellar pattern. Second, through formation of multiple, overlapping layers of cells, they generate a *tortuous*, intercellular *pathway* that impedes the egress of water [14]. In addition to providing the framework for the permeability barrier, corneocytes subserve several other critical functions; including *resistance* to *mechanical*

*insults*, as well as hydration and pliability, an additional set of functions related to the humidity-dependent hydrolysis of filaggrin into amino acids and their deiminated products (see below).

Another SC function that is universally impaired in the ichthyoses is desquamation. Normal desquamation is an invisible process, whereby single corneocytes at the skin surface are invisibly swept away. Normal desquamation represents an orderly process of loss of corneocyte cohesion, which is mediated by corneodesmosomes, intercellular protein connectors [15–17]. Corneodesmosome degradation is mediated by several proteases, and regulated by protease inhibitors and pH [18, 19]. In the predominantly retention hyperkeratoses (e.g., ichthyosis vulgaris), desquamation is the SC function that is primarily affected.

Due to the energy losses that accompany evaporative water loss, infants and children with severe phenotypes can exhibit growth failure due to increased caloric requirements [20, 21]. Other functional deficiencies include heat intolerance in patients with more severe generalized ichthyoses (e.g., lamellar ichthyosis), due to obstruction of sweat ducts. In certain ichthyoses there is also an increased susceptibility to cutaneous and systemic infections. Although not yet confirmed experimentally, a plausible scenario for the infectious complication is as follows: Certain SC lipids (e.g., free fatty acids) and anti-microbial peptides (defensins and cathelicidins) are lamellar body-derived residents of the SC intercellular domains that provide a first line of defense against microbial invasion (innate immune system). Failure of lamellar body secretion (e.g., EHK) or of lipid processing (required for generation of free fatty acids) (e.g., Netherton syndrome) or proteolytic inactivation of anti-microbial peptides (e.g., Netherton syndrome) may therefore account for the propensity for bacterial and fungal infections in EHK, as well as bacterial and viral infections in Netherton syndrome [22–24].

## “Lipid” versus “Corneocyte” Defect

Irregularities of epidermal lipid metabolism were among the first to be shown to cause disorders of cornification [25–29]. For example, lack of steroid sulfatase in recessive x-linked recessive ichthyosis directly results in altered lamellar membrane structure and function. In contrast, the inherent attenuation of corneocytes in avian [30] and mammalian skin [31] was not accompanied by gross barrier abnormalities. Hence, corneocyte structural proteins were initially thought to primarily provide mechanical resilience to the SC. Nevertheless, evidence from human studies suggests that even primary corneocyte protein abnormalities are accompanied by substantial barrier impairment [32–38]. Defects in the cornified envelope scaffold result in barrier abnormalities both in lamellar ichthyosis [32, 33, 39], and in loricrin keratoderma [37], supporting a critical *scaffold function* of the corneocyte for the permeability barrier. In contrast, mutations in keratins 1 or 10 in EHK provoke a barrier abnormality by interfering with lamellar body exocytosis (in this instance a cytoskeletal, rather than a scaffold abnormality) [35]. Furthermore, in Netherton syndrome, unopposed epidermal protease activity results in desmosomal degradation and compensatory acceleration of lamellar body secretion [36, 38]. The phenotypes vary rather widely in these entities, as do their pathogenic mechanisms. Yet, despite the primary defect affecting either corneocyte structural proteins or proteolytic enzymes impairing corneocyte-to-corneocyte adhesion, the permeability barrier abnormality in all instances is due to enhanced water movement *between* rather than *through* the defective corneocytes (Table 2).

## Transglutaminase 1-Deficient Autosomal Recessive Congenital Ichthyosis (TGM1-deficient ARCI)

### Clinical characteristics

The group of autosomal recessive congenital ichthyoses (ARCI) includes lamellar ichthyosis (LI) and nonbullous congenital erythroderma (CIE), and comprises a spectrum of phenotypes ranging from large dark plate-like scales and often little to no erythema (LI) to a fine, lighter

scaling pattern, often with prominent erythema (CIE), with many intermediate phenotypes. Patients with this spectrum of phenotypes present at birth encased in a shiny, coherent coating, the 'collodion membrane'. During the first post-natal weeks, as the hyperkeratotic membrane is shed, it is replaced by scaling and lichenification that involves the entire body including the intertriginous areas, palms, soles and scalp. In rare cases, the phenotype may be very mild ("self-resolving collodion baby") or focal [40]. Although there rarely is corneal involvement, patients can show marked facial tautness with severe ectropion.

### Molecular defect

At least one third of ARCI are caused by mutations in transglutaminase-1 (TGM1), on chromosome 14 [41–43], resulting in impaired cornified envelope crosslinking [44]. To date, over 50 different mutations have been identified in this gene. Some mutations are proposed to destabilize a hydrophobic pocket, distorting the active site of the enzyme, and resulting in loss of activity [45]. Transglutaminase-1 not only crosslinks proteins, but also sphingolipids to the cornified envelope [46, 47]. *Ex vivo* gene replacement of TGM1, followed by transplantation to SCID mice, has been successfully used on the ARCI phenotype [48]. Recent progress in delineating the genetic spectrum of ARCI has uncovered several additional gene mutations, many of which appear to disrupt epidermal lipid metabolism [5, 49–51]. A clear correlation between these genotypes and the spectrum of ARCI phenotypes has yet to be established.

### Consequences of TGM1 mutations for morphology and function

The epidermis in TGM1-deficient ARCI is acanthotic, often with a prominent granular layer. There is marked orthohyperkeratosis and occasional focal parakeratosis. On electron microscopy of the SC, the cornified envelopes are focally attenuated [32] and the resilience of corneocytes to boiling in sodium dodecyl sulfate (SDS) and dithiothreitol (DTT) is diminished [47, 52]. Moreover, prominent abnormalities in extracellular lipid structures are evident ultrastructurally, with truncation and fragmentation of extracellular lamellar membrane arrays in regions where the cornified envelope is attenuated [32], as well as minor abnormalities in spacing of lamellar bilayers on electron microscopy [33] and x-ray diffraction [53]. Functionally, these membrane structural abnormalities correlate with a modest increase in rates of TEWL under basal conditions [32, 33, 39, 53]. However, interpretation of prior TEWL data is difficult, because several of these studies were conducted before genotyping was available, i.e., it is uncertain if all ARCI genotypes exhibit similar barrier impairments. Nevertheless, in biopsies from patients with proven TGM1-deficient ARCI, increased movement of lanthanum tracer through the intercellular pathway of the SC has been demonstrated [32], confirming a permeability barrier abnormality. Although the lamellar membranes are fragmented and truncated in TGM1-deficient ARCI, the cornified lipid envelope, a lipid membrane cross-linked to the outer surface of the cornified envelope is preserved, and omega-hydroxy-ceramide content, the lipid species cross-linked to the cornified envelope is normal [32, 54]. This suggests that the barrier defect is not due to failure to form or attach a cornified lipid envelope, and indicates further that enzymes other than TGM1 also participate in omega-hydroxy-ceramide crosslinking. Thus, the extracellular lamellar membrane abnormalities are best explained by the lack of a proper scaffold, consistent with the putative role of the cornified envelope as necessary for organization of the extracellular matrix into lamellar bilayers (Fig. 4, Table 3).

## Loricrin Keratoderma

### Clinical characteristics

Loricrin keratoderma exhibits a characteristic, honeycomb-like, palmo-plantar keratoderma on the acral extremities including palms and soles; a generalized fine-scaling; hyperkeratotic knuckle pads on the dorsal surface of the fingers; and, often, constricting bands encircling the fingers and/or toes (pseudoainhum) [55–61]. While these features are similar to those of classic

Vohwinkel syndrome [62], loricrin keratoderma lacks the neurosensory deafness of classic Vohwinkel syndrome, and additionally it exhibits the generalized scaling described above. This distinct phenotype is also referred to as Vohwinkel syndrome with ichthyosis, Camisa variant of Vohwinkel syndrome, or Vohwinkel disease limited to the skin.

### Molecular defect

Loricrin keratoderma is caused by dominantly inherited mutations in the loricrin gene localized within the epidermal differentiation complex (EDC) encoded on chromosome 1q21. Loricrin is a glycine-serine-cysteine-enriched protein synthesized in the stratum granulosum, that along with profilaggrin, localizes to keratohyalin granules [63–65]. In the outermost granular cell, loricrin migrates to the cell periphery, where it is deposited beneath the plasma membrane, and cross-linked to several other cytosolic proteins (e.g., involucrin, small proline rich proteins [SPRRs], elafin, repetin, S100, and up to ≈20 others) by TGM1 to form the cornified envelope. In loricrin keratoderma the mutations result in elongation of the C-terminal domain of the loricrin protein. The mutant loricrin is misdirected to the nuclei of the granular layer, fails to reach the cornified envelope and is retained in the parakeratotic SC [55, 66].

### Consequences of loricrin mutation for morphology and function

In loricrin keratoderma, the epidermis exhibits epidermal hyperplasia, hypergranulosis, marked hyperkeratosis and parakeratosis, with characteristic roundish (rather than flattened) retained nuclei in the lower SC [58, 67]. Corneocyte-to-corneocyte cohesion is impaired, as demonstrated by ready removal of SC by tape stripping [37]. Functionally, SC hydration is markedly decreased in the hyperkeratotic/honeycomb-type skin sites, and basal rates of TEWL are increased. Disorganized lamellar bilayers together with penetration of the water soluble, electron-dense tracer, lanthanum, through the extracellular space beyond the stratum granulosum-SC junction indicate that the increased water loss occurs predominantly via extracellular domains [37]. Bilayer abnormalities occur adjacent to regions in the lower SC in which discontinuities and attenuation of the cornified envelope are present. However, cornified envelope dimensions partially normalize in the outer SC, apparently due to ongoing, compensatory incorporation of other cornified envelope peptides (Fig. 4). This normalization correlates with persistence of abundant calcium in the extracellular spaces of the SC (due to the defective barrier), where it is in the correct location to activate TGM1. A primary pathogenic role of the cornified envelope scaffold abnormality in loricrin keratoderma is underscored by the presence of a normal lamellar body secretory system, with largely unimpeded secretion of lamellar body contents, with a normal corneocyte lipid envelope, and normal bound omega-hydroxy-ceramide content [37]. In summary, the permeability barrier abnormality in loricrin keratoderma can be linked to a defective cornified envelope scaffold in the inner SC layers (Fig. 4). Thus, both TGM1-deficient ARCI and loricrin keratoderma share a common disease pathomechanism. In TGM1-deficient ARCI, the cornified envelope cross-linking enzyme is defective, while in loricrin keratoderma, its major substrate is deficient (Table 3).

## Epidermolytic Hyperkeratosis (EHK)

### Clinical characteristics

A characteristic feature in many EHK patients is a phenotypic shift from widespread blistering at birth to prominent hyperkeratosis during later life. Due to the blistering phenotype of the newborn, EHK was previously termed bullous congenital ichthyosiform erythroderma (BCIE) to distinguish it from the autosomal recessive, non-bullous ARCI group. Erythroderma is prominent in some patients, but absent in others. The hyperkeratosis in EHK often is generalized, and it may either involve or spare the palms and soles [68]. In the generalized variants, typically, involvement of flexural and extensor surfaces of large joints (elbows, knees) is accentuated, sometimes with massive hyperkeratosis, giving a peculiar, ridged appearance.

Flexural scales often become secondarily colonized by bacteria, producing a foul odor. Variants with particularly widespread and thick, porcupine-like (hystrix) hyperkeratosis (but lack of an early blistering phase) have been termed ichthyosis hystrix of Curth and Macklin. A milder variant of EHK, ichthyosis bullosa of Siemens (IBS), is characterized by peeling as well as annular, hyperkeratotic plaques preferentially over joints, shins and the periumbilical region. There are also localized forms, which are more limited to acral and flexural surfaces plus the palms/soles, or distributed in a linear pattern along the lines of Blaschko; i.e., as epidermal nevi. Epidermolytic hyperkeratosis strictly limited to palms and soles has been termed Vörner's palmoplantar keratoderma (PPK).

## Molecular defect

Mutations in keratin 1 (chromosome 12q13) and keratin 10 (chromosome 17q21-q22) have been identified as the cause of the classic generalized form of EHK [69 70–77]. Localized forms are due to chromosomal mosaicism [66, 78–83]. The offspring of patients with mosaic or nevoid variants of EHK due to K1 or K10 mutations are at risk for generalized forms of the disease. As many as half of all cases have no family history of the disease, indicating a high frequency of spontaneous mutations. The wide spectrum of clinical variability is predominantly between kindreds [84, 85]. In the milder IBS variant, mutations in another keratin expressed in the granular layer, K2e, have been identified. In families with the EHK limited to palms and soles (Vörner's) mutations in keratin 9 have been identified.

Keratinopathies are inherited as autosomal dominant traits, and function in a dominant-negative manner to disrupt the keratin filament network within the keratinocyte cytosol. The main components of these networks are the keratins, the most abundant proteins produced during the vectorial process of epidermal differentiation [86]. Typically, one acidic (type I) keratin heterodimerizes with one basic (type II) keratin and two such dimers are arranged in an antiparallel and staggered configuration (protofilament) [87, 88]. In turn, two protofilaments comprise a protofibril, of which four assemble to form the 10nm keratin intermediate filament. Within these filaments, the most abundant epidermal keratins, keratins 1 and 10, become linked to the cornified envelope (CE) [89–91]. In the disrupted state, keratin filaments retract from their attachments beneath desmosomal plaques, forming clumps or perinuclear shells [92]. Skin fragility due to mechanical trauma, which is most pronounced in neonatal EHK, is an expected consequence of mutations in keratin 1 or 10, analogous to the fragile skin phenotype of epidermolysis bullosa, where mutations in the basally expressed keratins, K5 or 14, produce cytoskeletal defects [93–95] and result in a mechanobullous phenotype.

## Consequences of K1/10 mutations for morphology and function

The histopathology of EHK is distinctive. Underlying a massive hyperkeratosis there is a characteristic degeneration of the upper epidermal cell layers, which stimulated coinage of the term "epidermolytic". Large basophilic intracellular deposits are present in the upper epidermal layers that resemble enlarged keratohyalin granules, but which instead are composed of clumped keratin filaments [96]. These changes are evident ultrastructurally in cases where the light microscopic features are mild and non-diagnostic. Whereas basal TEWL rates are elevated by approximately three-fold in EHK, recovery rates are faster than in age-matched control skin [35]. There is no defect either in the cornified envelope, or in the adjacent cornified lipid envelope; hence, a corneocyte scaffold abnormality does not explain the barrier abnormality. Although the histologic appearance of the upper nucleated cell layers suggests increased fragility as the cause of the barrier abnormality in EHK [97], this was not confirmed experimentally. Instead, the water-soluble tracer, colloidal lanthanum, penetrates through the extracellular compartment, with little evidence of tracer accumulation within corneocytes [35]. Thus, despite the fragility of nucleated keratinocytes in EHK, tracer neither enters the corneocyte nor permeates through the abnormal, *intra*cellular route. Increased *inter*cellular

permeability in EHK, instead, correlates with decreased quantities and defective organization of extracellular lamellar bilayers (Fig. 5), which in turn can be attributed to incomplete secretion of lamellar bodies from SG cells, resulting in many "entombed" lamellar body remnants within corneocytes. Yet, this secretory defect can be temporarily overridden, since after acute barrier disruption, a rapid release of preformed lamellar body contents is observed in conjunction with increased organelle contents in the extracellular spaces, thereby accounting for the accelerated recovery kinetics in EHK (Table 3). Loss of calcium from the outer SG cells is also observed in EHK following acute barrier disruption, which may provide the signal for the rapid exocytosis of lamellar bodies that results in accelerated repair kinetics [35]. Thus, the baseline permeability barrier abnormality in EHK can be attributed to impaired lamellar body secretion (resulting in deficiency of lamellar membranes and failure of lipid processing due to impeded delivery of lipid processing enzymes), rather than to corneocyte fragility or an abnormal cornified envelope scaffold, a defect that can be overcome in part by external applications of stimuli for barrier repair. The impaired lamellar body secretion at baseline could derive from disruption of the cytoskeletal framework, as has been shown in other secretory cells [98], suggesting further that the intermediate filament framework plays a role in lamellar body secretion. Although the impaired desquamation in EHK could also be related to a failure to deliver lamellar body-derived desquamatory proteases, this mechanism still needs to be investigated. Similarly, the increased infection rate may be due to failed delivery of lamellar body-derived antimicrobial peptides (Fig. 5).

The dramatic "phenotypic shift" in the neonatal period from a mechanobullous phenotype, where blistering from trauma predominates, to a hyperkeratotic phenotype is probably driven by post- natal environmental changes. Immersed in the aqueous, intrauterine environment, the fetus has a limited need for a highly competent permeability barrier; hence, the predominance of the "pure" keratinopathy-induced mechanobullous phenotype in the newborn with EHK. At birth, the need for a competent barrier becomes paramount and barrier repair homeostatic responses are initiated (through loss of extracellular calcium and cytokine release) with induction of epidermal hyperplasia. This is associated with expression of the wound healing keratins, K6 and K16 [99–101], and it also involves c-myc and 14-3-3 proteins [97]. Expression of keratins 6 and 16 has been observed in both human EHK [83, 102], and in mouse models of EHK [69, 103–105]. Down-regulation of keratins 1 and 10 under conditions of epidermal hyperplasia and/or substitution of the unaffected hyperproliferative keratin pairs in keratin filament formation, may result in amelioration of the blistering in the mature EHK phenotype [106].

## Ichthyosis vulgaris

### Clinical characteristics

Ichthyosis vulgaris represents the most common disorder of cornification. The phenotype is rarely evident before 3–6 months after birth, but usually manifests during childhood and becomes more severe with age. Its prevalence appears to vary with geographical region. The highest prevalence was estimated to be up to 1 in 250 in British school children with dry skin [107]. In contrast, studies from Japan [108] and Mexico [109] estimated lower prevalences (i.e., 1: 1000 and 2000). Individuals with ichthyosis vulgaris commonly display rather mild generalized fine scaling with flexural sparing. The extensor surfaces of the extremities are usually most affected. Involvement of palms and soles (hyperlinearity) is common as is the association with keratosis pilaris and atopic dermatitis. It is difficult clinically to differentiate between mild ichthyosis vulgaris and the xerosis that accompanies atopic dermatitis, which is not surprising in view of the common mutations found in these disorders (see below). Varying clinical criteria for the diagnosis and/or geographical variation in the mutation frequency may account for the discrepant prevalence rates reported in the literature.

## Molecular defect

Common genetic variants in the gene encoding for filaggrin (*FLG*), located in the epidermal differentiation complex (EDC) on chromosome 1q21 [110, 111], have recently been proposed to cause ichthyosis vulgaris [112–117]. This was long suspected, because Sybert et al. had demonstrated that profilaggrin and its proteolytic product, filaggrin, are reduced or absent in ichthyosis vulgaris, correlating with instability of the profilaggrin message [118] and a long-recognized paucity in keratohyalin within the stratum granulosum [119120]. The delay in identifying the genetic basis of the FLG deficiency in ichthyosis vulgaris was due to its exceptionally long and highly repetitive gene sequence. Interestingly, even in the absence of overt mutations, there is a range from 10–12 FLG repeats within the gene, variability which may account for more subtle phenotypic differences [121], and it could further explain the discrepant prevalence data (see above). In IV, there is a dose effect wherein heterozygous patients display a mild or no phenotype, while homozygous and compound heterozygous *FLG* genotypes exhibit the full ichthyosis vulgaris phenotype. The same *FLG* mutations have been linked to atopic dermatitis [113, 117], but these are predominantly found in the heterozygous configuration. Overall, the high prevalence of *FLG* mutations even in phenotypically normal subjects suggests there may be an evolutionary advantage conferred by FLG deficiency.

Profilaggrin is the initial product of translation of the *FLG* gene. It is found in the keratohyalin granules in the outer nucleated layers of the epidermis, which are responsible for the designation of this layer as the granular layer. Profilaggrin is a large, histidine-rich, highly cationic phosphoprotein, consisting of filaggrin repeats, connected by peptide segments enriched in hydrophobic amino acids [64, 122]. Profilaggrin is both dephosphorylated and proteolytically processed during cornification. Immunolocalization studies suggest that processed FLG peptides also associate with the cornified envelope [123]. Above the stratum compactum, FLG is deiminated and proteolyzed further into its constituent amino acids and other small molecules [124–126], whose hygroscopic properties underlie SC hydration [127]. Their generation is catalyzed by an aspartate protease whose activity is upregulated when relative humidity levels decline <80%, as occurs normally in outer SC [128]. In contrast to the cytoplasmic location of the C-terminal FLG monomers, the N-terminal portion of profilaggrin is found in the nucleus consistent with its nuclear localization sequence (S100-like EF-hand), which may indicate a calcium-dependent nuclear function [129].

## Consequences of FLG mutations for morphology and function

A histologic feature of ichthyosis vulgaris is a reduced or absent granular layer with a paucity of keratohyalin granules and reduced cellular profilaggrin content [120]. Absence of the granular layer is independent of body site and season of the year, but correlates with severity of the disease [120], and mutational status. Patients with one mutation (heterozygous) display a reduced granular layer whereas patients with two *FLG* mutations (homozygous or compound heterozygous) in most instances show a virtually complete lack of a granular layer [112, 115, 116]. However, in rare cases, even patients with two mutations still show residual granular cells [130]. This appears to depend on the location of the mutation within the gene; i.e., more proximal mutations show complete absence of profilaggrin in homozygotes [112], whereas more distal mutations show residual, yet greatly reduced, truncated profilaggrin species that are not processed to FLG monomers [117]. On electron microscopy, residual keratohyalin granules have been described as poorly formed ("crumbly") [131] or absent [120]. Although initially FLG monomers were believed to mediate the collapse of the keratin filament network during cornification, the fact that the keratin intermediate filaments are normal in ichthyosis vulgaris [119, 132] suggests that FLG is not required for keratin aggregation. Therefore, the pathogenesis of the scaling abnormality in ichthyosis vulgaris remains to be resolved. One possible explanation is that a reduction in proteolytic FLG breakdown products results in a lack of

osmotically active small molecules that regulate corneocyte hydration [125, 133, 134]. In support of this hypothesis, FLG hydrolysis has been shown to be regulated by environmental conditions with increased hydrolysis under conditions of xeric stress [127]. In ichthyosis vulgaris, corneocytes would have reduced capacity to imbibe water when exposed to high humidity or during bathing. This could impair desquamation, since outer corneocytes would not "swell and slough" with the friction that accompanies bathing. As FLG has been proposed to participate in the linkage of keratins to the cornified envelope during cornification [123], ichthyosis vulgaris could also be another example of a scaffold abnormality [135]. Alternatively, it is also possible that other properties of the FLG degradation products play a pathogenetic role in ichthyosis vulgaris, e.g. FLG degradation contributes to stratum corneum pH, which is an important determinant of permeability barrier function (Fig. 6, Table 3).

## Netherton syndrome

### Clinical characteristics

Patients with Netherton syndrome (syn. ichthyosis linearis circumflexa, Comèl-Netherton syndrome) display generalized scaling with marked inflammatory features, often with similarities to atopic dermatitis. At or shortly after birth, infants commonly present with generalized erythroderma. In older children and adults, a unique scaling pattern, termed ichthyosis linearis circumflexa, may develop in which serpiginous areas of double-edged scale are present. Patients with Netherton syndrome are at risk for excessive systemic absorption of topical medications as a result of a severe barrier abnormality. The skin changes are typically accompanied by pathognomonic hair shaft defects, called bamboo hair or trichorrhexis invaginata, in which the distal hair segment is telescoped into the proximal part. Only 20–50% of hair may be affected, and this feature may be absent in some patients. Atopy with anaphylactic reactions to food is another feature of the disease.

### Molecular defect

Netherton syndrome is caused by autosomal recessive mutations in the *SPINK5* gene, coding for the serine protease inhibitor lymphoepithelial-Kazal-type 5 inhibitor (LEKTI), and presumably resulting in unopposed serine protease activity. A large number of different mutations have been documented in the literature, many of them abolishing enzyme activity, a few mutations merely compromising enzyme function [136–139]. LEKTI is expressed in skin, mucous membranes, tonsils and thymus [140, 141]. It is cleaved into 15 active domains that suppress proteases. However, the specific biological targets of LEKTI in human tissues are unknown. Among possible targets are the stratum corneum trypsin- and chymotrypsin-like enzymes (SCTE and SCCE, respectively) [18, 142–145], whose defective inhibition by LEKTI could result in both premature corneodesmosome degradation with premature desquamation as well as in the inactivation of other proteins, such as lipid hydrolases required for processing of lamellar body lipids. Other putative targets are trypsin-like serine proteases, including the membrane-type serine protease 1 (MT-SP1). Thus, LEKTI is believed to play a role in corneocyte desquamation, as also suggested by its restricted expression in the granular layer of the epidermis, and the severe cornification abnormality in Netherton syndrome. LEKTI may also be important in the down regulation of inflammatory processes, since the phenotypic finding of atopic manifestations in Netherton syndrome is not seen in other congenital ichthyoses, and polymorphisms in the *SPINK5* gene (missense variants) have been associated with atopic disease [146].

### Consequences of SPINK5 mutations for morphology and function

Histological analysis shows acanthosis and a thin, variably parakeratotic SC in Netherton syndrome. The histological picture can be very similar to psoriasis, particularly in infants. Perivascular inflammation may be present, and neutrophils have been described to invade the

epidermis. On electron microscopy, multilocular vesicles filled with an amorphous substance have been described at the level of the stratum spinosum [147–150], which correlate with premature secretion of lamellar body contents [38]. The lamellar body-derived extracellular lamellae are disturbed and interspersed with foci of electron dense material [38]. Overall, SC thickness is reduced [151]; hence in Netherton syndrome both the quantity and quality of SC membranes are abnormal (Fig. 5) [20].

We showed recently that the severity of the cutaneous phenotype in Netherton syndrome (including the barrier abnormality) correlates inversely with both the extent of LEKTI protein reduction and with the increase in serine protease activity [36]. Unrestricted serine protease activation results in loss of SC due to accelerated desmosomal degradation. In contrast to a loss- of- function mutation of *SPINK5* in transgenic knock out mice, which results in early post-partum death [152], most humans with Netherton survive, probably due to compensatory, suprabasal upregulation of desmosomal proteins and accelerated lamellar body secretion [36, 38]. Nevertheless, the secreted contents of lamellar bodies are not processed into functional lamellar bilayers in Netherton syndrome due to proteolytic degradation of lipid-processing enzymes (Fig. 7, Table 3).

Growth failure is a common complication for infants with Netherton syndrome, and it is attributed predominantly to a severe barrier defect with loss of calories through heat of evaporation [20, 21]. Infants with Netherton syndrome are at risk for systemic infections, with significant morbidity and mortality, which maybe a result of proteolytic attack on SC antimicrobial peptides. The antimicrobial peptides, cathelicidin and human β-defensin, SC serine proteases, and their inhibitor, LEKTI, all co-localize to lamellar bodies [153–155]. Indeed, a disturbed regulation of antimicrobial peptide (cathelicidin) proteolysis has been linked to LEKTI deficiency in the mouse model [156]. There is also an increased susceptibility to cutaneous HPV infections in Netherton syndrome, and cases of HPV-associated, non-melanoma skin cancer have been reported [24, 157–160]. Yet, it is not known if these predispositions are related to disturbances in SC innate immunity or to the T-cell abnormalities associated with the atopic state.

## Conclusion: Pathways to Abnormal Cornification and Towards a Function-Driven Model of Disease

While it has been useful to categorize the ichthyoses into disorders primarily affecting corneocyte proteins (“the bricks”) vs. those due to lipid metabolic defects (“the mortar”), the complex structure of tissue and functional interdependencies of SC constituents render this simplistic scheme unsatisfactory. The intercellular matrix contains important structural proteins, the corneodesmosome components, as well as enzymes that modulate SC functions, and antimicrobial peptides. Moreover, it is apparent from studies of defects of corneocyte proteins that competent SC extra-cellular membranes and lamellar body secretory system require a structurally preserved corneocyte. Thus, all of the ichthyoses due to corneocyte abnormalities studied to date provoke a defect in the extracellular lamellar membranes and generate a permeability barrier abnormality with accelerated transcutaneous water movement via the SC interstices. Abnormal permeability barrier function drives compensatory manifestations, including epidermal hyperplasia and hyperkeratosis. As the SC serves as a biosensor, transmitting danger signals to the underlying nucleated, epidermal cell layers, any acute or sustained insult that results in barrier impairment stimulates homeostatic repair responses, including both increased synthesis and secretion of lamellar body lipids and a mitogenic stimulus to the epidermis. Hence, the hyperkeratosis in most ichthyoses is largely the result of a hyperproliferative response to the permeability barrier abnormality in these disorders in an attempt to normalize barrier function. Ultimately, the hyperplastic response provides both more corneocytes as ‘bricks’, and more keratinocytes that synthesize lipids

('mortar') for the barrier. The second major functional disturbance is abnormal desquamation. The gradual weakening of intercellular connections, through regulated proteolysis of corneodesmosomes and, perhaps, assistance of mechanical debridement by corneocyte hydration leading to normal, invisible, single cell desquamation is altered in the ichthyoses. Development of a function-driven model of pathogenesis, while still a work in progress, will provide a rational classification of this diverse group of genetic disorders that share the similar clinical phenotype of ichthyosis. It can be integrated with prior histometric and morphological ("the bricks and mortar") models of disease and provides insights into mechanisms of disease and potential complications. Most importantly, it can guide the development of rationale therapeutic approaches [161, 162]

## Acknowledgements

Editorial Comment:

Over the past 15 years, there have been significant advances in our understanding of the disorders of cornification. Currently, there are more than 25 identified genes involved in the pathogenesis of the ichthyoses. This diverse group of disorders presents many diagnostic as well as therapeutic challenges to the clinician. Unfortunately, advances in treatment lag far behind the exponential growth in the genetic understanding of these diseases.

In this update, Drs. Schmuth, Gruber, Elias and Williams masterfully review the role of corneocyte proteins in the disorders of cornification using a new, function-driven model of pathogenesis. They explain how the effect of corneocyte mutations on barrier function have enhanced our understanding of the critical interactions between corneocytes and the intercellular lamellae in a normal permeability barrier.

Furthermore, their function-driven model provides a framework for understanding how such diverse genetic alterations can result in similar phenotypic features among the numerous disorders of cornification. Despite the many pathogenetic origins of impaired permeability barrier, patients with these conditions share compensatory responses of epidermal hyperplasia and hyperkeratosis.

The authors have contributed greatly to our understanding of the ichthyoses over the years. They are sure to enlighten you with this outstanding update.

Amy Jo Nopper, MD

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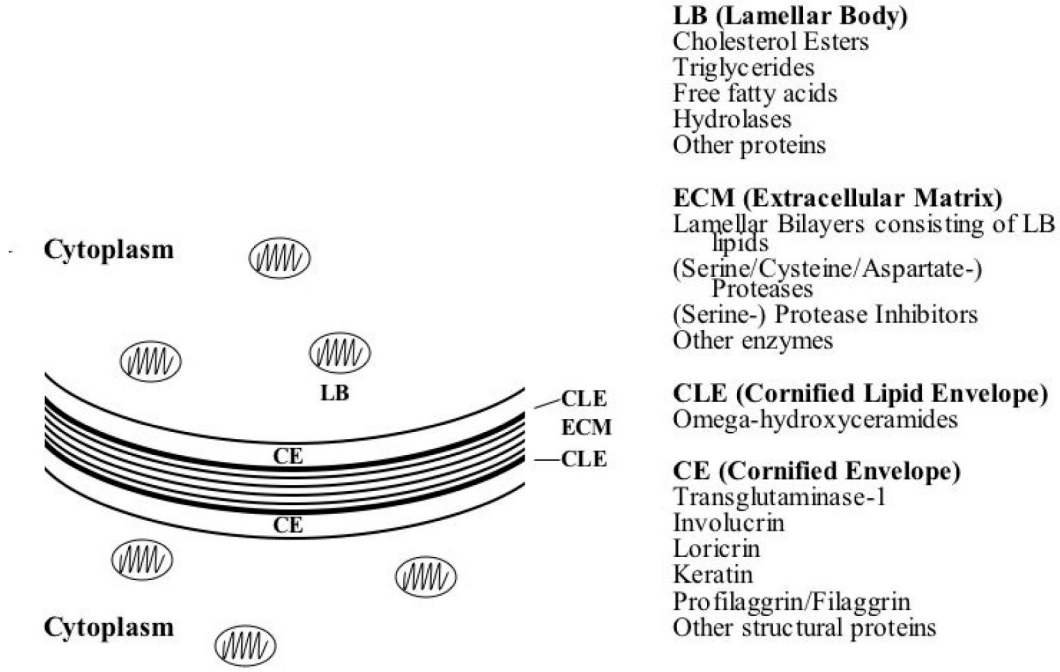


**Figure 1. Key stratum corneum components**

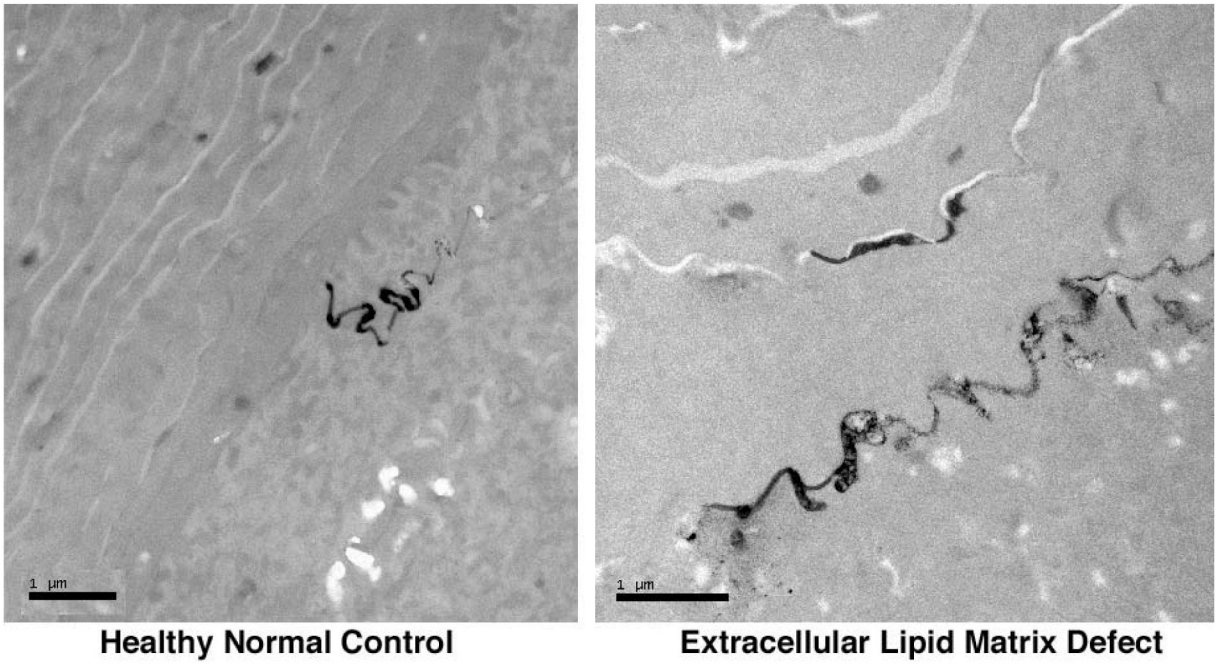


**Figure 2. Lanthanum penetration through the extracellular pathway**

In normal control skin (left panel), electron dense lanthanum tracer permeation is halted at the stratum granulosum – SC junction, whereas in ichthyosis vulgaris (right panel), tracer permeates beyond the junction, but remains restricted within the extracellular lamellar lipid pathway. Freshly obtained skin biopsies were exposed to 4% colloidal lanthanum nitrate tracer in 0.05 M Tris buffer, pH 7.4, for one hour, containing 2% glutaraldehyde and 2% paraformaldehyde, followed by post-fixation in $OsO_4$. Magnification bars = 1 µm (left panel), and = 2 µm (right panel).

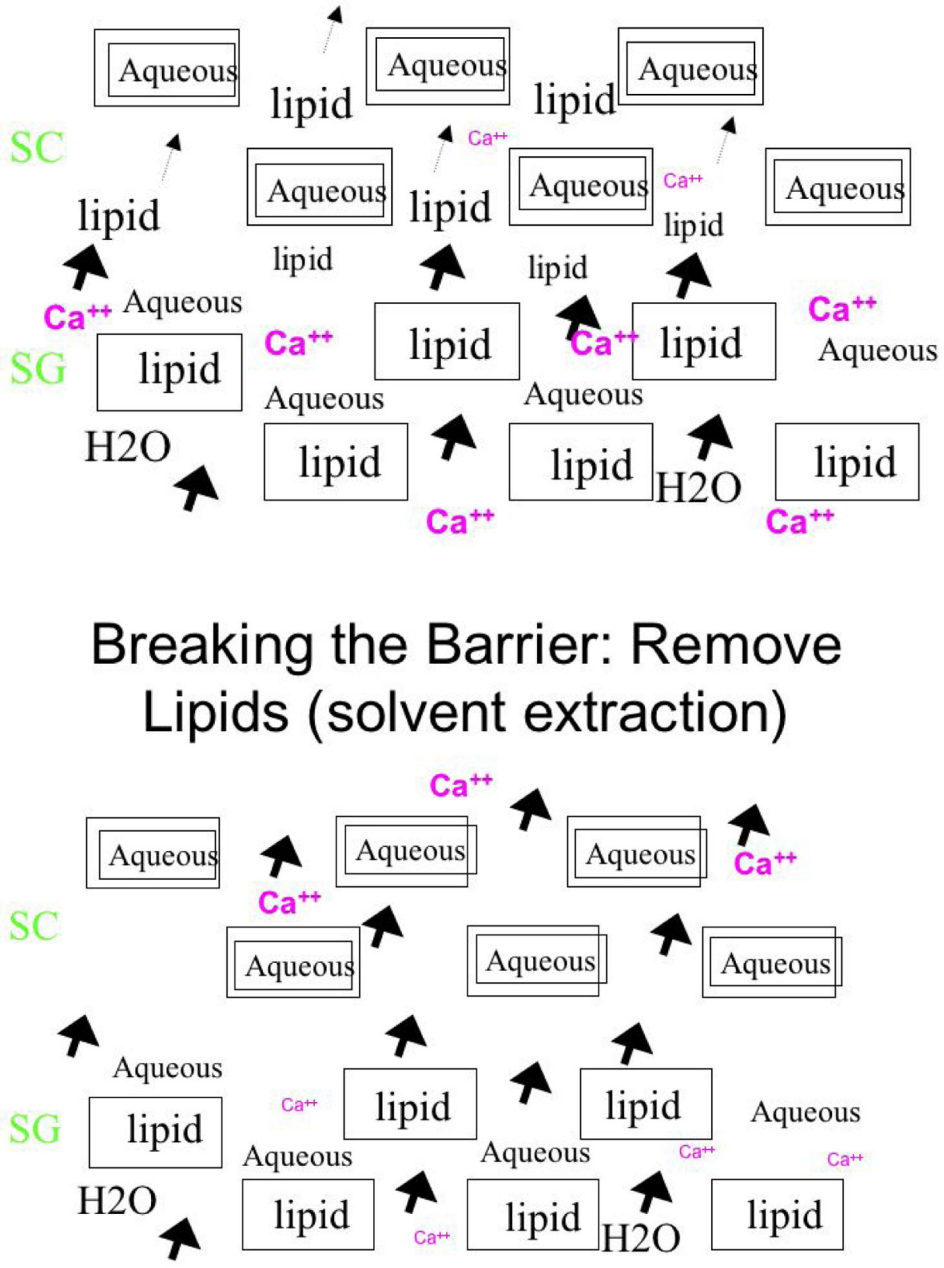


**Figure 3. Two compartment model of SC**

In normal control skin (upper panel), the calcium ion gradient peaks in the lower SC, whereas after barrier disruption via solvent extraction of extracellular matrix lipids (lower panel) there is a rapid loss of the SC calcium gradient.

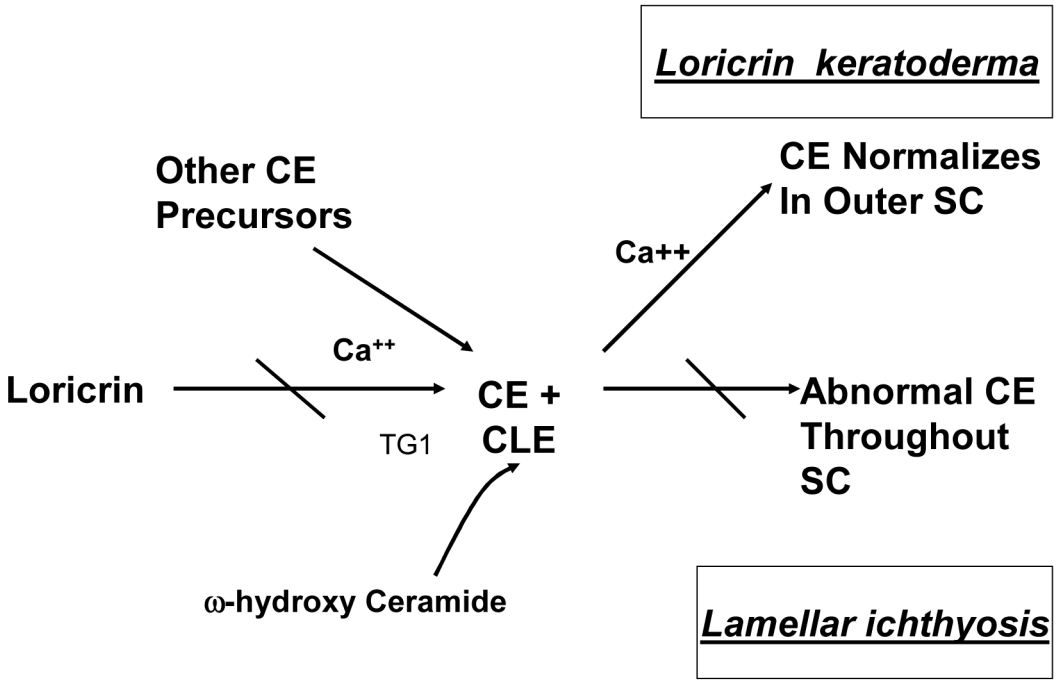


**Figure 4. Scaffold abnormalities in lamellar ichthyosis and loricrin keratoderma**

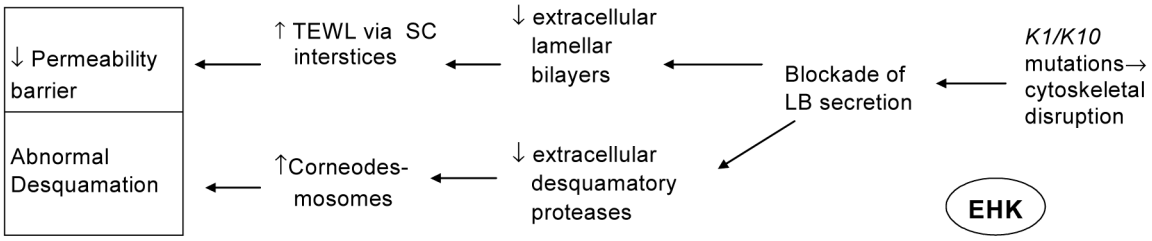


**Figure 5. LB secretory defect in EHK**

While the overall number of LB is normal in EHK, arrays of individual organelles remain restricted beneath the apical plasma membrane of outermost granular (SG) cells (A, B, arrowheads). C: Lack of LB secretion results in a paucity of extracellular deposits at the SG-SC interface (asterisks). $RuO_4$ post-fixation. Magnification bars = 0.5 μm.

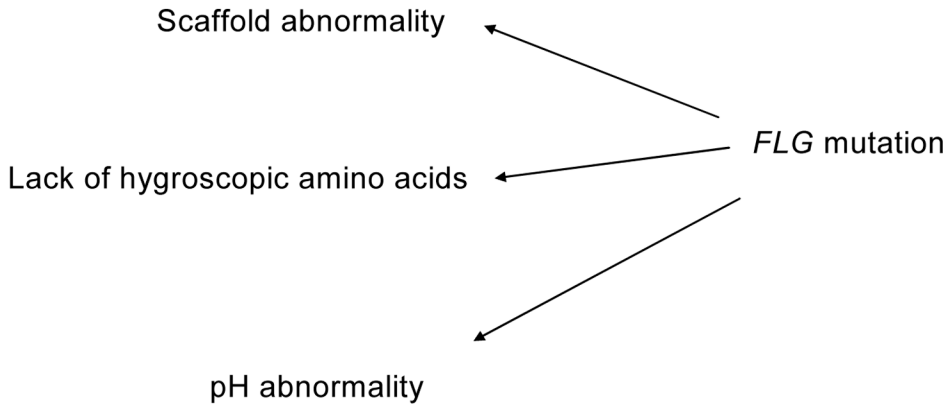


**Figure 6. Possible mechanisms for barrier abnormality in ichthyosis vulgaris**

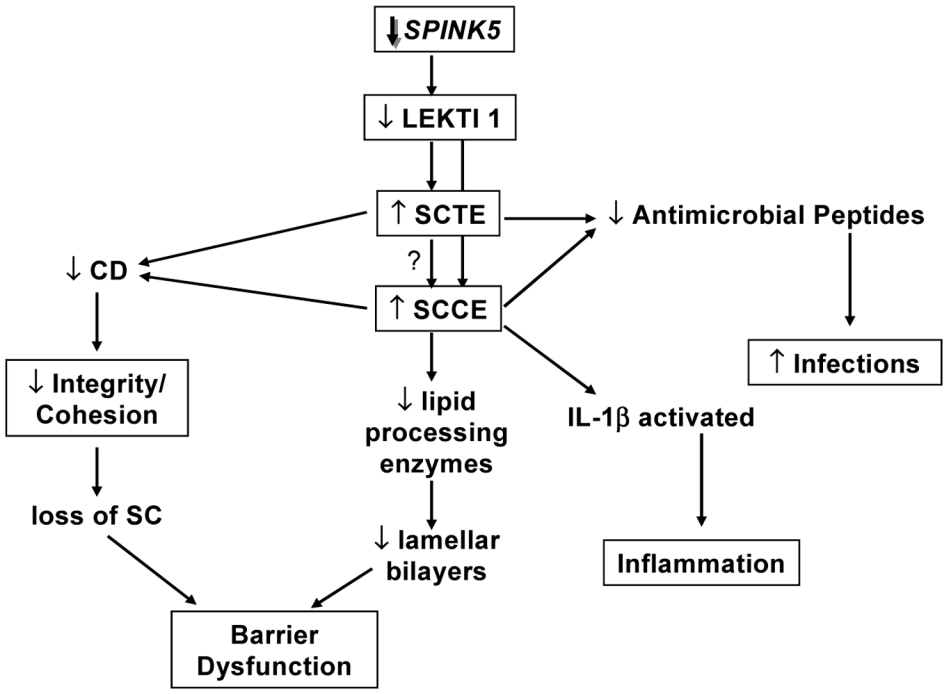


**Figure 7. Pathogenesis of Netherton syndrome**

**TABLE 1**

Pathogenesis of Ichthyoses: Corneocytes vs. Extracellular Lipid Matrix

1. Ichthyosis is a final common pathway for errors in genes expressed in the epidermis
2. Genes encoding a wide spectrum of epidermal proteins have been shown to cause ichthyosis
3. Impaired stratum corneum barrier function results from divergent mechanisms depending on the underlying gene mutation
4. Although diverse molecular defects underlie the various subtypes, ichthyoses are characterized by a stereotypic epidermal response with acanthosis, hyperkeratosis, and extracellular permeability barrier abnormalities

**Table 2**

Principles of a Function-Driven Model of Disorders of Cornification (with Examples)

1. **The Permeability Barrier is abnormal** to varying degrees in all ichthyoses. Barrier defects may arise due to:
   - **a.** **Abnormal lipid composition of intercellular membranes leading to lamellar/nonlamellar phase separation**
     - i. **Inborn errors of lipid metabolism** resulting in over- or under- representation of key SC lipids (i.e., cholesterol, free fatty acids, ceramides) that form the lamellar membranes and/or the introduction of other lipid species. (**X-Linked I.; NLSD; Refsum disease, ARCI-Lipoxygenase pathway**)
     - ii. **Failure of lipid processing** to key SC lipids due to:
       - **1.** **Deficiency of lipid processing enzymes**
         - a. Loss of function mutations (**Neonatal Gaucher D.**)
         - b. Failure of lamellar body delivery (**EHK, CEDNIK Syndrome**)
         - c. Proteolytic attack (**Netherton**)
       - **2.** **Increase in SC pH** leading to inhibition of lipid processing enzymes with acidic pH optima (?**I. vulgaris**)
   - **b.** **Paucity of lamellar membranes** due to failure of:
     - i. Lamellar body organellogenesis (**Harlequin I.**)
     - ii. Lamellar body secretion (**EHK; CEDNIK syndrome**)
   - **c.** **Abnormal cornified envelope scaffold** for organization of lamellar membranes (T**GMI-def. ARCI; Loricrin K.**)
2. **Homeostatic responses attempt to repair the barrier defect.**
   - **a.** **These responses include:**
     - i. increased epidermal lipid synthesis
     - ii. epidermal hyperplasia
     - iii. inflammation
   - **b.** Because of the genetic defect, the barrier defect cannot be repaired and these homeostatic responses are sustained.
   - **c.** The severity of the barrier defect determines the intensity of the homeostatic repair responses.
   - **d.** Environmental conditions modify the barrier defect.
     - i. The need for a permeability barrier in the aqueous, *in utero* environment is much reduced.
     - ii. At birth, the xeric stress of post-natal life augments the barrier repair homeostatic responses. In ichthyoses with severe barrier defects, this stress results in a striking “phenotypic shift” (**Harlequin I; EHK; Collodion baby phenotypes**).
3. Desquamation is also impaired in all ichthyoses. Abnormal desquamation can be mediated by:
   - **a.** **Delayed or accelerated corneodesmosome proteolysis**
     - i. Protease inhibition (**X-linked I.**) or failure of protease delivery leads to delayed corneodesmosome degradation (**Harlequin I; EHK**)
     - ii. Loss of protease inhibition leads to accelerated corneodesmosome degradation (**Netherton**)
   - **b.** **Epidermal hyperplasia with incomplete terminal differentiation**
     - i. Entombed lamellar bodies in hyperplastic corneocytes with failure of protease delivery (**Hyperplastic ARCI phenotypes**)
     - ii. Elevated SC pH inhibiting proteases with acidic pH optima (**?I. vulgaris**)
   - **c.** **Decreased corneocyte hydration** with loss of hydration-related corneocyte swelling exerting mechanical stress on intercellular connections
     - i. Deficiency of filaggrin breakdown products (**?I. vulgaris**)

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**Table 3**

Function-Driven Model of Generalized Disorders of Cornification: Defects of the Corneocyte Proteins

| Disorder | 1° Defect | Downstream effect | SC Function | Homeostatic response |
|---|---|---|---|---|
| TGM1-def. ARCI & Loricrin K. | Fragile cornified envelope | Abnormal scaffold for lamellar membranes | ↑↑ TEWL | ↑↑ Epidermal hyperplasia |
| Epidermolytic HK | Disrupted keratin | Impaired lamellar body secretion | ↑↑ TEWL<br>↓↓ CD digestion | ↑↑ Epidermal hyperplasia<br>↓↓ Desquamation |
| Ichyosis vulgaris | ↓ Filaggrin | ↓↓ Corneocyte hydration<br>↑ SC pH (?) | ↓↓ Hydration | ↓ Desquamation |
| Netherton syndrome | Disrupted corneocyte adhesion | Abnormal scaffold | ↑↑ CD digestion | Epidermal hyperplasia ↑↑ Desquamation |