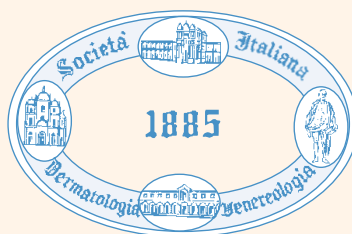


GIORNALE ITALIANO DI

DERMATOLOGIA E VENEREOLOGIA

OFFICIAL JOURNAL OF THE SOCIETÀ ITALIANA DI DERMATOLOGIA MEDICA,
CHIRURGICA, ESTETICA E DELLE MALATTIE SESSUALMENTE TRASMESSE (SIDeMaST)



VOLUME 148 - No. 1 - FEBRUARY 2013

E D I Z I O N I M I N E R V A M E D I C A

Netherton syndrome and its multifaceted defective protein LEKTI

M. D'ALESSIO ¹, P. FORTUGNO ², G. ZAMBRUNO ¹, A. HOVNANIAN ^{3, 4}

Netherton syndrome (NS, OMIM 256500) is a rare autosomal recessive disorder manifesting with congenital ichthyosis, a specific hair shaft abnormality named trichorrhexis invaginata, and atopic manifestations. Because of severe complications frequently occurring in the neonatal period, NS prognosis can be poor in infancy. NS is due to loss-of-function mutations in the *SPINK5* gene and to the consequent lack of expression of its encoded protein LEKTI in the skin and all stratified epithelial tissues. Following the identification of the NS causative gene and protein, specific diagnostic tools have been developed, thus breaking up the challenge of distinguishing NS from other congenital ichthyoses with overlapping features, and from severe, early-onset forms of atopic dermatitis or psoriasis. Intensive efforts to extend the knowledge into the pathomechanisms of NS have also been made. However, NS management is still problematic due to the lack of specific treatment and unmet needs. This overview summarizes the current state of the art in NS research with an emphasis on the progress made toward disease-specific innovative therapy development.

KEY WORDS: Netherton syndrome - *SPINK5* - Serine proteases - Inflammation.

Netherton syndrome (NS, OMIM 256500) is a rare and severe genodermatosis firstly described in the mid 1950s by the Italian dermatologist, Marcello Comèl and, a few years later, by the American colleague Earl Weldon Netherton, as a peculiar disease, because of the concurrence of a congenital ichthyosiform dermatosis and a specific hair shaft abnormality named “bamboo hair” or trichorrhexis invaginata (TI).^{1, 2} Congenital generalized erythema,

¹Laboratory of Molecular and Cell Biology
Istituto Dermopatico dell'Immacolata
IDI-IRCCS, Rome, Italy

²Dermatology Unit, Bambino Gesù Children's Hospital
IRCCS, Rome, Italy

³Université Paris V René Descartes
Sorbonne Paris Cité, INSERM U781
Imagine Institute, Paris, France

⁴Department of Genetics
Necker Hospital for Sick Children, Paris, France

together with scaling and peeling of the skin, TI, and constant and severe allergic manifestations represent the distinctive features of NS (Figure 1). Hypernatremic dehydration, hypothermia, recurrent bacterial infections and weight loss are frequent during the neonatal period and, in the absence of specific treatment, can lead to a fatal course.^{3, 4} NS is a monogenic disorder inherited as autosomal recessive trait with an incidence estimated at 1/200,000 births and no difference among sex or ethnic and racial groups.⁵ The NS defective gene, *SPINK5* (serine protease inhibitor Kazal-type 5), encodes LEKTI (lympho-epithelial Kazal-type related inhibitor), a protease inhibitor strongly expressed in the skin and all stratified epithelia.⁶⁻⁸ Based on different lines of evidence, including careful examination of *Spink5* knockout mice (KO) and other animal models, NS manifestations can be mainly referred to a severe alteration of the skin barrier function.⁹⁻¹² In spite of the significant progress in molecular genetics of NS over the past decade, no specific treatment for NS is currently available. Quite recently, however, new innovative approaches have been developed, some of

Corresponding author: G. Zambruno, Université Paris V René Descartes, Sorbonne Paris Cité, INSERM U781, Imagine Institute, Paris, France.

which may potentially lead to treatment of NS at the molecular level. By inferring the translational implications of the data collected over the last sixty years or so of research in NS, we here summarize the most relevant up-to-date information on NS etiopathogenesis, diagnosis and management.

NS clinical presentation

In infants and children

The classical presentation of NS at birth or within the first days of life is of an infant with generalized erythroderma and scaling (Figure 1A).¹³ However, there is a wide variation in the intensity and extent of redness and peeling of the skin.^{3,4} In particular, some patients will show continuous sheets of peeling skin and erythroderma, while others will display only minor scaling and/or redness of the skin. Collodion baby is very unusual in NS. Lesions will often evolve into the typical pattern of ichthyosis linearis circumflexa (ILC) made of serpiginous migrating plaques with a double scaling edge (Figure 1B).¹ Persistent involvement of the face with demarcated erythematous patches affecting preferentially the cheeks is typical (Figure 1C). Generalized erythroderma often subsides with age and more localized lesions appear. Severe pruritus is common and causes irritability and sleeplessness. Hair is often sparse or absent at birth, but may be initially normal. Eyebrows and eyelashes are also frequently affected. The hair classically remains sparse, thin, fragile and slow growing (Figure 1C-E,G). Hair light microscopy examination shows trichorrhexis invaginata (TI), with a ball-and-socket appearance corresponding to the invagination of the distal hair shaft into its proximal part (Figure 1F).^{2,14} Dermoscopic examination of hairs, eyebrows or eyelashes can also be useful for visualizing TI.^{15,16} TI is specific for NS and almost always present after the age of one year. "Golf tee hairs" result from broken hairs with TI.^{17,18} Trichorrhexis nodosa and pili torti can also be seen, but are not specific for NS.

Atopic disease is a major feature of NS. Eczematous-like patches (Figure 1C,E), which are distinct from those of atopic dermatitis (AD), are associated with allergic asthma, urticaria, angioedema and allergic rhinitis.^{19,20} Several patients also develop lichenified and hypertrophic lesions (Figure 1G,H). Food allergies are very frequent, in particular to nuts,

egg and milk, with elevated specific immunoglobulin E (IgE) levels. Hypereosinophilia is common and high serum IgE levels are almost constant.^{3,4}

During infancy, NS patients are at high risk of severe life-threatening complications, in particular hyponatremic dehydration and recurrent cutaneous and systemic bacterial (*e.g.*, *Staphylococcus aureus*), mycotic and viral infections.²¹⁻²⁵ Hyponatremic dehydration is caused by the huge increase in transepidermal water loss (TEWL) consequent to the skin barrier defect which is particularly severe in infancy. Failure to thrive is also a very frequent problem early in life, to which chronic diarrhoea, recurrent infections, food allergy and malnutrition can contribute. As a result, growth retardation and short stature are common features of NS.^{3,4} Several rare complications have been described, often in individual cases, including neurological complications secondary to dehydration, staphylococcal scalded skin syndrome, acute pancreatitis, acute bilateral renal vein thrombosis, rickets, and persistent pulmonary hypertension.^{23,26-31}

In adults

The clinical presentation varies with age and often partially improves in adults. The disease most often has a fluctuating course, with flares of "eczematous-like" skin inflammation (Figure 1I) and/or ILC (Figure 1B). Hairs are classically rare, fragile, spiky, but there is a considerable variation between patients. Hair can have a normal appearance, although TI is usually present. Allergic manifestations can also be very different from patient to patient. Lichenification of the popliteal and antecubital fossae is frequent. Papillomatous skin lesions are sometimes seen. Of note, occurrence of squamous cell carcinomas, HPV-positive papillomatous skin tumours and giant condyloma of Buschke-Löwenstein has been reported in individual cases, which questions whether NS could predispose to the development of these tumours.³²⁻³⁶

NS diagnostics

As already mentioned, a precocious diagnosis represents a very important issue for NS patient management. However, in some instances, it may be very challenging. In fact, even if the concomitant presence of ichthyosiform erythroderma since infancy, TI and atopic diathesis, is highly suggestive of NS (Figure



Figure 1.—Netherton syndrome clinical and diagnostic features. A) Congenital scaly erythroderma in a NS newborn; B) detail of typical lesions of ichthyosis linearis circumflexa (ILC); C) eczematous-like red and inflammatory plaques on the face of a child affected with NS; note the almost absent eyebrows; D, E) sparse, fragile scalp hair and almost absent eyebrows and eyelashes in a child affected with NS; note the eczematous lesions of AD of the face and the scaling of the scalp; F) bamboo-like hair shaft (TI); G, H) highly inflammatory and lichenified plaques on the scalp and trunk of a child affected with NS; note the almost complete absence of scalp hair; I) inflammatory skin lesions in an adult patient with NS; L) NS skin histopathology showing non-specific or “psoriasiform” changes, with acanthosis, hyperkeratosis and parakeratosis. The presence of a mild inflammatory perivascular infiltrate in the papillary dermis is also visible; M; N) immunohistochemical analysis of LEKTI expression in normal (M) and NS patient skin (N) showing the complete absence of LEKTI expression in the epidermal granular layer of NS patient skin (N), as compared to the control (M).

1A-I), lack or delayed appearance of any of these features may occur. In addition, also ILC, which is very evocative of NS, may be delayed, atypical or totally absent. Finally, in adults, the variable expression of the skin lesions and their resemblance with those present in patients affected by severe forms of AD or psoriasis may further jeopardize NS diagnosis. Routine histopathological examination of skin biopsies does not increase the likelihood of a prompt and accurate NS diagnosis. In fact, NS skin displays non-specific or "psoriasiform" changes, with hyperplasia of the subcorneal epithelium (acanthosis) and elongation of the rete ridges, reduction and focal absence of the granular layer, hyperkeratosis, persistence of nuclei in the corneocytes of the stratum corneum (SC) (parakeratosis) (Figure 1L). Intracellular or extracellular oedema and spongiosis of the lower epidermal layers can also be observed. Finally, a mild to moderate inflammatory infiltrate with eosinophils and mast cells can be present in the papillary dermis (Figure 1L).

That said, until the discovery of the NS defective gene and protein,⁷ erythrodermic infants with NS were often misdiagnosed as having severe generalized seborrheic dermatitis (previous Leiner's disease), acrodermatitis enteropathica, a severe form of psoriasis, or a recessive disorder of cornification such as congenital ichthyosiform erythroderma (CIE), or inflammatory peeling skin syndrome (type B). By year 2003, instead, the availability of antibodies specifically directed against the NS defective protein LEKTI, has made feasible to specifically diagnose NS.⁸ Indeed, the demonstration of defective LEKTI expression in the granular layer of the epidermis and in the inner root sheath of the hair follicle is highly specific for NS (Figure 1M,N). Some rare NS patients, often less severely affected, show markedly reduced, but still positive, LEKTI staining. However, in all other infantile erythrodermas LEKTI immunostaining of skin sections is either positive or, like in psoriasis, can be distinguished from that of NS patients because of the positive staining of the inner root sheath of the hair follicle.^{8, 37}

To date, the diagnosis of NS can also be supported by the identification of the genetic mutations in the LEKTI encoding gene, *SPINK5*.^{7, 12, 23, 31, 38-58} Whenever appropriate, the search for the disease-causing mutations should be proposed in the context of genetic counselling in that it makes feasible the DNA-based prenatal diagnosis for couples with

an affected child. This is now performed at 10-12 weeks of gestation by foetal DNA analysis isolated from chorionic villus sampling.^{40, 41} Reference centres for NS molecular diagnosis and genetic counselling are scattered all over the world and can be easily retrieved from Orphanet (<http://www.orpha.net>), NCBI GeneTest (<http://www.ncbi.nlm.nih.gov/sites/GeneTests>) or other web portals.

Molecular genetics

NS is caused by loss-of-function mutations in *SPINK5*, a single copy gene comprising 33 exons spanning 61 kb on chromosome 5q32 (Figure 2A).⁷ Since its first discovery,⁷ more than 70 different *SPINK5* mutation types have at present been reported (Figure 2B) (<http://www.hgmd.cf.ac.uk/ac>).^{12, 23, 31, 38-58} Nonsense, frame-shift, and splice-site mutations are equally represented and spread all over the gene. Among *SPINK5* mutations, several hot-spot mutations, possibly favoured by the presence of stretches of repeated nucleotides, have been observed in different haplotypes (*e.g.*, 2468_2469insA^{7, 12, 39, 42, 48}). In addition, founder mutations have been identified in patients originating from Greece (c.891C>T)⁴⁷ and Turkey (c.153del1).³⁹ In particular, c.891 C>T is a synonymous mutation compatible with residual LEKTI expression and has recently been shown to represent the most frequent cause of NS in Southern Europe.⁴⁷ Thus, knowledge on the family geographic origin is most useful to facilitate mutation identification. In most NS patients, the *SPINK5* causative mutations lead to premature termination codons and, because of nonsense-mediated mRNA decay, result in complete absence of detectable LEKTI protein. However, residual LEKTI protein, capable to mitigate the disease phenotype, has occasionally been reported.^{12, 52, 53} Of interest, among the NS patients with no detectable LEKTI, a quite considerable variability in the disease phenotype has been observed.^{12, 47, 52, 53} These data, together with the observation of intrafamilial differences,⁴⁷ suggest that other genetic, epigenetic and/or environmental factors contribute to the NS phenotype. As it will be elucidated in the next paragraphs, the NS pathogenic mechanisms are quite complex in that LEKTI is directly or indirectly involved in several biological cascades relevant to skin homeostasis (*i.e.*, SC desquamation, cornified envelope formation, lipid permeability barrier matu-

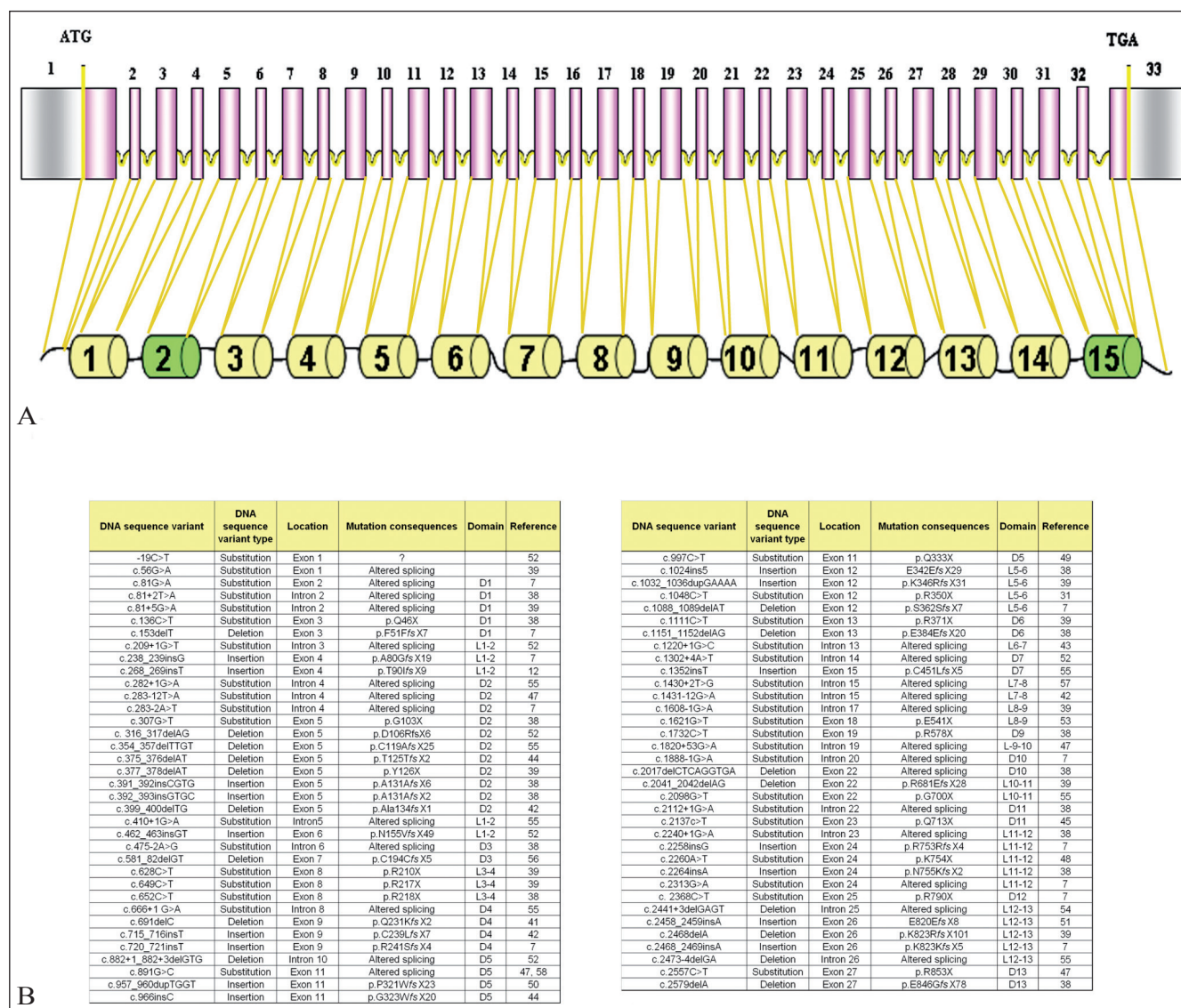


Figure 2.—Molecular basis of NS. A) Schematic representation of the *SPINK5* gene and its encoded protein LEKTI. *SPINK5* comprises 33 exons spanning 61 kb on chromosome 5q32. It encodes for LEKTI, a serine protease inhibitor composed of 15 domains (D1-D15) interspaced by linker regions (L1-2-L14-15). Within the LEKTI sequence, only two of the potential inhibitory domains (2 and 15, in green) contain 6 evenly spaced cysteines responsible for the 3 intramolecular disulphide bonds characteristic of Kazal-type related inhibitors. The remaining domains (in yellow) contain 4 cysteines. (B) NS associated *SPINK5* genetic mutations, as listed in the literature. For each mutation the DNA mutation type, its consequences at protein level, its location on LEKTI protein, and the reference relative to the first publishing are indicated. Nucleotide numbering refers to the cDNA sequence with nucleotide position 1 assigned to the first nucleotide of the ATG initiation codon in exon 1.

ration, antimicrobial defence, inflammation and allergy) (Figure 3). Thus, reasonably any other gene acting in the same pathways could act, at any time, as modifier and modulate the severity of the skin barrier defect and/or its consequences on inflammation

and allergy. Unraveling these modifiers would represent an important step towards the understanding of biological networks regulating protease activation and their effects on the epidermal barrier formation and turnover, as well as on the immune system.

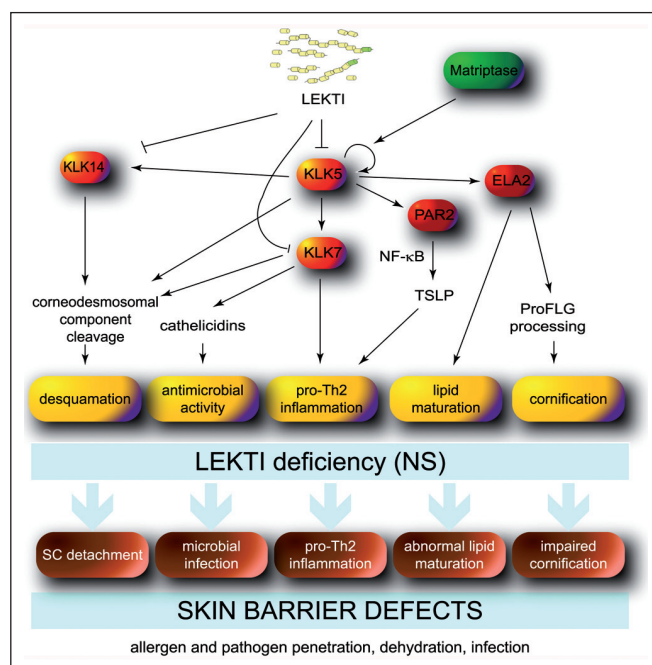


Figure 3.—Model for NS pathogenesis and LEKTI functioning in the skin. In the skin the absence of LEKTI has the following major consequences: 1) the unopposed KLK activities degrade the corneodesmosomal components and lead to SC detachment from the granular layer; 2) the overactive KLK5 activates ELA2 whose enhanced activity accelerates Pro-FLG proteolytic processing and results in abnormal organization and composition of intercorneocyte lipid lamellae. Both these processes cause a severe skin barrier defect that leads to the release of endogenous danger signals. In parallel, 3) the KLK5 hyperactivity also causes PAR2 activation, which triggers a proallergic and proinflammatory cascade via the NF- κ B pathway. Overexpression of TSLP and secretion of IL-8, TNF- α and other proinflammatory cytokines represent the ultimate outcome of this pathological mechanism. Finally, 4) LEKTI defective expression is thought to alter the activity of antimicrobial peptides processing enzymes, thus affecting the innate immune defence of the skin.

Animal models

The identification of *SPINK5* as the NS causative gene came out as a surprise.⁷ In fact, at that time the role of the *SPINK5* encoded protein LEKTI in skin biology had not yet been recognized. To decipher the LEKTI function(s) and to examine the consequences of its defective expression, *Spink5* KO murine models were developed. The *Spink5* deficient mice reproduced the NS phenotype and displayed abnormal desquamation, impaired keratinisation, hair malformation and a severe skin barrier defect.⁹⁻¹¹ Newborn mice showed severe peeling of the skin as-

sociated with a dramatically enhanced TEWL that leads to death of the pups by dehydration within 10 hours. Biochemical and ultrastructural analyses of skin extracts from the *Spink5* KO mice revealed that LEKTI deficiency causes loss of adherence of the SC with abnormal desmosome cleavage in the upper granular layer. This anomaly associates with excessive degradation of desmoglein 1 (Dsg1) and other desmosomal components,¹⁰ likely consequent to lack of inhibition of serine proteases involved in the physiological SC desquamation process. Accordingly, epidermal samples from newborn *Spink5* KO mice showed enhanced proteolytic activities of KLK5 (stratum corneum tryptic enzyme, SCTE) and KLK7 (stratum corneum chymotryptic enzyme, SCCE), two major epidermal serine proteases able to degrade desmosomal proteins.^{10, 59, 60} In addition, overactivity of a new serine protease, subsequently recognized as elastase 2 (ELA2), was observed.^{10, 61} Thus, the *Spink5* KO murine model provided *in vivo* evidence that LEKTI is a key regulator of protease activities in the epidermis. These results disclosed a major disease mechanism involving the degradation of desmosomal components by unrestrained KLK5 and KLK7 activity and leading to premature SC detachment and a severe skin barrier defect.⁹⁻¹¹

The keratinization process was also altered and profilaggrin (Pro-FLG) processing was enhanced.^{10, 11} Finally, lipid droplets were present in corneocytes of the newborn pups and intercorneocyte lipid lamellae were abnormal.¹⁰ These data indicate that the protease unregulation consequent to LEKTI defective expression distress several aspects of skin barrier homeostasis.

As to the development of allergy in NS patients, it could have been inferred that increased penetration of allergens and microbes through impaired barrier is the underlying mechanism. However, other ichthyoses with a skin barrier defect do not develop allergies, and it was thus hypothesized that a specific defect was underlying the propensity of NS patients to present atopic manifestations. Because newborn *Spink5* KO mice die a few hours after birth, the skin from *Spink5* KO embryos at day 19.5 was transplanted onto nude mice to follow the phenotypic changes of the mutant mice skin after prolonged exposure to the environment.¹⁰ Five weeks after transplantation, the skin grafts developed large scales and marked erythema without any erupted hair shafts. Histological examination of the engrafted skin showed

NS-like features with an acanthotic epidermis with papillomatosis, prominent hyperkeratosis, focal parakeratosis and hypogranulosis.¹⁰ These epidermal alterations were also associated with a moderate to severe perivascular inflammatory infiltrate in the papillary dermis, a feature reminiscent of NS skin. In this experimental set up with a protected environment devoid of allergens and microbes, the activation of a proinflammatory cytokine cascade by the LEKTI deficient cells represented a solid rationalization. To explore the molecular mechanisms leading to the recruitment of inflammatory cells in NS skin, *Spink5* KO embryos and the graft model of transplanted *Spink5* KO skin were therefore evaluated for the expression levels of proinflammatory cytokines.⁶² Both the *Spink5* KO embryos and the transplanted skin displayed increased expression of proinflammatory mediators, which induces inflammatory cell infiltrates containing mast cells and eosinophils, and abnormal levels of pro-Th2 chemokines.⁶² These results indicate that LEKTI defective epidermis activates a proinflammatory cascade independently of the primary epithelial failure and the environment. Remarkably, by using double KO mice for *Spink5* and the protease-activated receptor-2 (*Par2*), Briot *et al.* demonstrated that the activation of the inflammatory process in NS skin can also be mainly traced back to KLK5 hyperactivity secondary to the LEKTI defective expression.^{62, 63} Indeed, KLK5 hyperactivity activates a proallergic signalling cascade that results in overexpression of the allergic and inflammatory mediators ICAM1, IL-8, TNF- α and TSLP. In particular TSLP is a major pro-Th2 cytokine overexpressed in AD skin.⁶⁴⁻⁶⁶ This pathway involves the activation of specific receptors (*i.e.*, PAR2) present at the cell surface of keratinocytes, upon their cleavage by KLK5. Notably, this pathway is detectable during embryonic life of *Spink5* KO mice as soon as 19.5 days, thus it is initiated in the absence of any challenge by allergens or microbes.^{62, 63} Finally, a further study conducted in *Spink5*-deficient mice showed that LEKTI, together with KLK5 and 7, and possibly other serine proteases, participates in the control of the innate immune defence through the regulation of the *in vivo* processing of the antimicrobial peptide cathelicidin.⁶⁷ In NS, all these mechanisms add up to the skin inflammation process, involving the release of IL-1 β driven by the tissue injury and keratinocyte stress consequent to SC detachment.

Aside from *Spink5* KO mice, other murine mod-

els have also been crucial to dissect the pathogenesis of NS. In particular, transgenic mice overexpressing KLK7 in suprabasal epidermis developed epidermal thickening, hyperkeratosis, dermal inflammation and severe itching.^{68, 69} These results supported the role of KLK7 in several features of the NS disease phenotype. Transgenic KLK5 mice confirmed the pivotal role of KLK5 in NS pathogenesis.⁷⁰ Finally, ELA2 transgenic mice (Tg-ElA2)⁶¹ displayed a considerable reduction of the keratohyalin granules, abnormal Pro-FLG processing, and impaired lipid lamellae structure, which are both observed in *Spink5* KO mice. These observations suggest that ELA2 participates in both pro-FLG cleavage and SC lipid metabolism. Interestingly, *in vitro* studies showed that LEKTI does not inhibit ELA2 and that the increased elastase 2 activity observed in NS murine models results from unrestrained KLK5 proteolytic activity, instead. Indeed, KLK5 activates ELA2 by cleaving its inactive precursor pro-ELA2.⁶¹ In conclusion, these different mice models have implicated KLK5, KLK7 and ELA2 in the pathogenesis of NS. Interestingly, ablation of matriptase from LEKTI-deficient mice dampened inflammation, eliminated aberrant protease activity, prevented detachment of the SC, and improved the barrier function of the epidermis.⁷¹ These results uncovered the role of matriptase in a pro-KLK pathway central in NS manifestations. However, matriptase is not directly involved in NS pathogenesis since it is not inhibited by LEKTI and is not overexpressed in *Spink5* deficient mice.⁷¹

NS pathomechanisms

The results obtained in murine models have unveiled the biological cascades involved in NS thus orientating patient biological investigations.¹² Similarly to *Spink5* KO mice, NS patients show SC detachment with (corneo)desmosomal cleavage between the granular layer and the first corneocyte layer, concomitant with reduced Dsg1 and desmocollin 1 (*Dsc1*) expression in the uppermost living layers of the epidermis and premature degradation of corneodesmosomes.¹² As observed in *Spink5* KO mice, increased activity of KLK5 and KLK7 into the cell layers displaying Dsg1 and *Dsc1* immunostaining reduction, is also present. These results strongly indicate that premature degradation of corneodesmosomal structural components, secondary

to unopposed KLK5 and KLK7 activities, is a major pathomechanism of NS.¹² Similarly to *Spink5* KO and *Ela2* transgenic mice, NS patients also show significant alteration of the epidermal differentiation process.^{10, 12, 61} Persistent presence of nuclei in corneocytes, premature secretion of lamellar bodies, altered expression of early and late differentiation markers are seen at all times. In addition, NS epidermis displays a considerable reduction of the keratohyalin granules which correlates with decreased pro-FLG immunostaining.¹² As indicated by Tg-Ela2 *in vitro* and *in vivo* studies, these abnormalities result from the increased proteolytic activity of ELA2 which participates to pro-FLG cleavage.⁶¹

In light of these results, uncontrolled KLK activity in NS secondary to LEKTI defective expression affects three major aspects of skin biology, i.e. epidermal differentiation, barrier formation and SC shedding, all converging in a severe skin barrier dysfunction.⁷² Still, as we have learned from the *Spink5* KO mouse models, an additional mechanism involving PAR2, and leading to the production of the major pro-Th2 cytokine TSLP and other proinflammatory mediators, accounts for the severe inflammatory and allergic manifestations that are constant in NS, but are not seen to a similar extent in other inherited ichthyoses. Indeed, Briot *et al.* showed that NS patients display overexpression of TSLP in epidermis, likely because of unopposed KLK activity, whereas TSLP is not detected in healthy controls.⁶² In the same study, by showing that NS keratinocytes in culture display a significant increase in TSLP expression, compared to control keratinocytes, Briot *et al.* also proved that TSLP production represents an intrinsic property of *SPINK5*^{-/-} keratinocytes.⁶² In cultured NS keratinocytes, ICAM1, IL-8 and TNF- α were also significantly enhanced but, while downregulation of KLK5 by siRNA drastically reduced TSLP expression, a less significant decrease of IL-8 and TNF- α , and no effect on ICAM1 expression were seen.⁶² These results correlate, for the first time, LEKTI deficiency with KLK5 hyperactivity, TSLP upregulation and NS allergic manifestations. Still, they demonstrate that KLK5 induces the production of other inflammatory molecules that favour a pro-Th2 microenvironment. Finally, these events occur as a cell-autonomous process elicited by LEKTI deficiency.⁶² Thus, we can conclude that a protease signalling, mainly mediated by KLK5, is responsible not only for the severe epidermal barrier defect, but

also for the skin inflammatory process and allergic manifestations observed in NS. In addition, the recent observation by Bennet *et al.* that NS skin displays an unusual expression pattern of the antimicrobial peptide dermcidin,⁷³ together with the abnormal expression of cathelicidin observed in *Spink5* KO animals,⁶⁷ suggests a direct LEKTI involvement also in the innate immune defence. To interpret this data the authors postulated an appealing hypothesis which functionally links the abnormal processing of dermcidin peptides with the high susceptibility of NS patients to recurrent staphylococcal infections. Specifically, they proposed that dermcidin, which is constitutively expressed in human sweat glands and transported via sweat through the epidermis, is proteolytically activated by the neutrophil serine protease cathepsin G, upon its secretion in response to infectious stimuli. On the epidermal surface LEKTI controls this proteolytic activity.⁷³ Similarly, the high susceptibility to *S. aureus* infection and colonisation of AD patients has been linked to reduced amounts of dermcidin-derived peptides in their sweats.⁷⁴

These studies aimed at decipher the NS pathomechanisms have taught us that KLK activity regulation represents an essential aspect of skin biology. This regulation takes place at different levels. In fact, at first KLKs are expressed as inactive pro-forms which require proteolytic cleavage for protein activation. This is achieved through a complex mechanism with auto- and cross-activation steps involving various KLKs as well as unrelated proteases. After that, KLK proteolytic activities are tightly controlled by a finely tuned interaction with serine protease inhibitors.⁷⁵ Crucial among the latter is certainly LEKTI, whose inhibitory activity is required in various instances. Based on NS patients and animal studies, the following model for NS pathogenesis and LEKTI functioning in the skin can be proposed (Figure 3). In the epidermis, the absence of LEKTI results in unrestrained activity of KLK5, KLK7 and likely other KLKs (*i.e.*, KLK14). As a consequence, premature degradation of corneodesmosomal components, and thus detachment of the SC from the granular layer, occur. In parallel, the overactive KLK5 activates ELA2 whose enhanced activity accelerates Pro-FLG proteolytic processing and results in abnormal organization and composition of intercorneocyte lipid lamellae. Both these processes cause a severe skin barrier defect that not only causes dehydration, but also facilitates allergen and pathogens pen-

etration and leads to the release by the mechanically stressed keratinocytes of pro-IL-1b and other endogenous danger signals. In NS patients, KLK5 hyperactivity secondary to LEKTI deficiency, also causes PAR2 activation, which triggers a proallergic and proinflammatory cascade via the NF- κ B pathway (Figure 3). The ultimate outcome of this additional pathological mechanism primed by LEKTI defective expression is represented by the overexpression of the major pro-Th2 cytokine TSLP (Figure 3). This cytokine activates Langerhans cells, promotes Th2 differentiation of naive T cells and stimulates secretion of IL-8, TNF- α and other proinflammatory cytokines, which contribute to the recruitment of inflammatory cells (eosinophils and mast cells) in the skin. Finally, several data indicate that LEKTI defective expression also affects the activity of antimicrobial peptides and thus the innate immune defence of the skin.^{67, 73}

The NS defective protein LEKTI, a multifaceted protein

LEKTI is a serine protease inhibitor strongly expressed in all stratified human epithelia, including the skin, the oral and genital mucosa where it is expressed in the most superficial layers.⁸ LEKTI is thus expressed at the front side of the body defence. Interestingly, LEKTI is expressed in the thymus, a lymphoid organ which is essential for T cell education and immune tolerance.^{6, 8} LEKTI is not detectable either in haematopoietic cells, or in the lung and the gastrointestinal tract,⁸ but proteolytic fragments are detected in the blood circulation, suggesting a possible role at distance.⁶

From what we have learned thus far, LEKTI is a pleiotropic serine protease inhibitor participating as regulator to a number of biological pathways relevant to skin permeability barrier, hair morphogenesis, inflammation and allergy.⁷⁵ This competence relies on several aspects of its biology. At first, as member of the serine protease inhibitor Kazal-type family, LEKTI has a very peculiar primary structure in that is composed by as many as 15 potential inhibitory domains (D1-D15) (Figure 4).⁶ These proteins, characterized by the presence of three conserved disulfide bonds within the reactive site loop, are, in fact, usually composed of only one inhibitory domain. In the second place, LEKTI undergoes through both

post-transcriptional and post-translational modifications which contribute to the generation of an ample number of fragments with related but distinct structure and role.^{76, 77} In human, following alternative pre-mRNA processing, *SPINK5* encodes 3 LEKTI isoforms differing in their C-terminal portion: the most abundant LEKTI Full-Length with 15 domains (LEKTI_{FL}, 145-kDa), a Long isoform (LEKTI_L, 148-kDa) carrying a 30 amino acid residue insertion between D13 and D14, and a Short isoform (LEKTI_{Sh}, 125-kDa) composed of the first 13 domains.⁷⁶ All LEKTI isoforms contain a signal peptide and the inhibitory domains are separated by linker regions (Figure 4). In the skin, LEKTI is at first synthesized as precursor proteins by differentiated keratinocytes of the upper spinous/granular layers.⁸ Then, LEKTI precursors are subjected to sequential cleavages by furin in the postendoplasmic reticulum compartment to generate several single or multi-domain polypeptides (Figure 4).^{8, 77} Immunoelectron microscopy studies have shown that LEKTI is transported in lamellar granules, in compartments distinct from KLK5 and KLK7, and that LEKTI fragments are secreted in the extracellular space at the granular layer/SC interface.⁷⁸ There they exert their inhibitory function interacting with serine proteases, among which the most relevant are the KLKs. Finally, progressive SC acidification mediates the release of the active enzyme from LEKTI fragments,^{78, 79} allowing the serine protease functioning. By combining antibody mapping, N-terminal sequencing, and site-specific mutagenesis, Fortugno *et al.* defined the aminoacid sequence of most of the LEKTI polypeptides physiologically generated in human epidermis and drew the activation cascade model depicted in Figure 4 for LEKTI_{FL}.⁷⁷ By studying the inhibitory specificities/activities of the identified LEKTI fragments toward the epidermal serine proteases KLK5, KLK7, and KLK14, they also proved that all KLKs tested were inhibited by LEKTI polypeptides with Ki in the nanomolar range. In agreement with the major increase of trypsin versus chymotrypsin-like activity observed in SC of NS patient,⁵³ the strongest inhibition was achieved toward KLK5 and KLK14, in particular by fragments derived from the LEKTI region D6-D9.⁷⁷ The same polypeptides were also very effective inhibitors of KLK-mediated DSG1 degradation, a key step in corneocyte shedding. These results were consistent with several previous *in vitro* studies^{60, 79} and formally proved that each one of the LEKTI

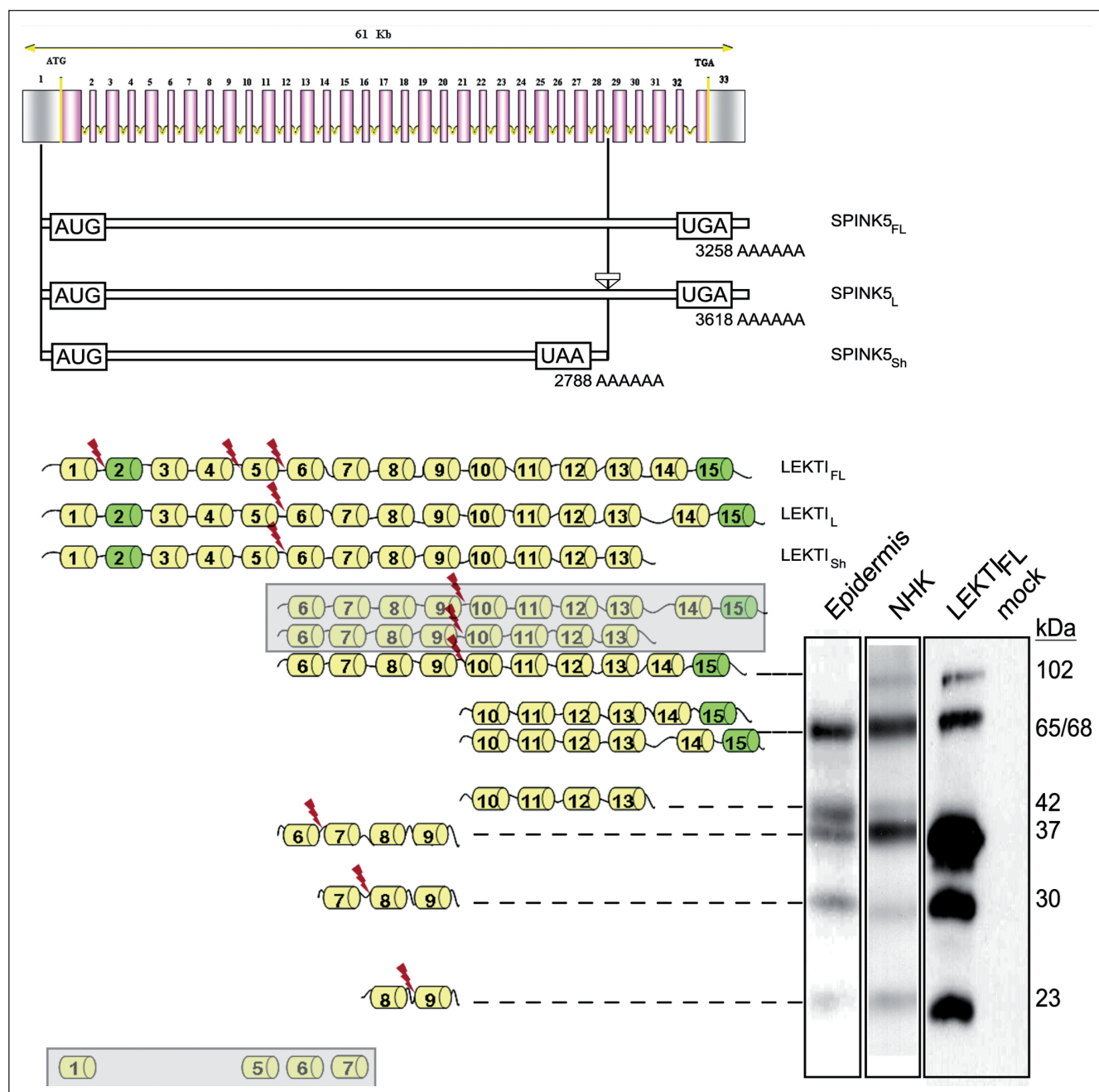


Figure 4.—From *SPINK5* to LEKTI inhibitory fragments: a complex path. The *SPINK5* gene and corresponding mRNAs (upper panel). Schematic representation of the encoded *LEKTI*_{FL}, *LEKTI*_L and *LEKTI*_{Sh} isoforms (middle panel) with location of the furin cleavage sites used for LEKTI post-translational processing. On all LEKTI isoforms, a furin driven proteolytic cascade generates a discrete number of single and multidomain, at time overlapping, fragments, as shown in the lower panels. Detection of LEKTI fragments in normal human epidermal samples, conditioned media from differentiated normal human keratinocytes, and conditioned media from HEK293 transfected with p*LEKTI*_{FL} or the empty vector, by immunoblotting with the anti LEKTI D7-D12 polyclonal antibody (on the right). The identities of LEKTI fragments, as determined by antibody mapping, N-terminal sequencing, and site-specific mutagenesis, and the corresponding molecular weight (in kDa) are indicated on the left. The LEKTI portions not detected by the anti D7-D12 are grey shadowed.

polypeptides physiologically generated in the epidermis displays a specific inhibitory profile towards epidermal proteases. Interestingly, Fortugno and colleagues showed that, at the site of their potential interaction, these LEKTI fragments and KLK5 are present in a nearly 1:1 molar ratio, which is essential for a fine-tuned inhibition.⁷⁷

NS clinical management and therapy

Being a multisystemic disorder with life threatening complications, NS early management requires a multidisciplinary approach by a specialized team. The treatment of patients with NS is usually conservative and symptomatic. In the neonatal period, it is important to control temperature instability and excessive transcutaneous fluid loss, the hypernatremic dehydration that may ensue, and to treat infections and other complications. Hospitalization in intensive care units is usually required for monitoring of body temperature, water-electrolyte balance, infections and other organ involvement. The skin manifestations are treated by regular application of emollients; therapeutic intervention for infections with topical and systemic antibiotics is the mainstay. Skin inflammation is difficult to treat at this age, as topical steroids expose to systemic side effects due to the severe skin barrier defect. Pruritus and irritability are common and treatment with antihistamines can be attempted. Appropriate nutritional support is crucial and will preferably use hypoallergic diet and milk substitutes. Gastrointestinal feeding can be required. Careful monitoring of growth by a dietician and nutritionist is essential to prevent malnutrition and, as far as possible, failure to thrive during early ages.

Overtaken these critical urgencies, clinicians ought to consider that the disease symptoms can vary between patients and even within the same patient over time. Therefore, it is important to tailor therapy to the individual NS patient. Oral retinoids have been reported to lead to disease worsening. Topical calcineurin inhibitors (tacrolimus and pimecrolimus), which have been demonstrated to improve the skin findings and quality of life of patients with severe chronic dermatoses including AD, have recently been used in NS, but their effects are controversial. Owing to the underlying skin barrier dysfunction, the possibility of significant systemic absorption and resultant adverse events represents a real concern when these

agents are used in patients with NS. Indeed, in spite of a considerable improvement of the clinical condition, significant percutaneous absorption of the drug, with serum levels well above the therapeutic range, have been reported in three patients treated with 0.1% tacrolimus ointment twice daily.⁸⁰ Other studies described effective treatment with careful monitoring of blood levels.^{81, 82} The treated area should remain below 20% of the body surface. As to pimecrolimus few reports refer of both clinical efficacy and good clinical tolerability with a reduced percutaneous penetration and lower blood levels.⁸³⁻⁸⁵ Topical steroids are usually more effective than calcineurin inhibitors. However, they expose NS patients to a significant risk of systemic penetration due to altered permeability barrier,⁸⁶ and it is recommended to restrict their use. Recently, intravenous immunoglobulin (IVIg) treatment repeated every 4 weeks showed remarkable efficacy in five children.⁸⁷ Skin inflammation, itching and infections significantly improved, as well as the overall quality of life, height and weight curves. The precise mechanism by which IVIg improves NS is not known, but it could involve compensation of a functional immune defect. Phototherapy, in particular narrow band UVB and UVA1, has been reported to be beneficial in a few patients,^{88, 89} but its usefulness awaits confirmation and prolonged treatment should be avoided due to possibility of development of squamous cell carcinomas in NS patients.³⁴ Finally, anti-TNF- α antibody (infliximab) therapy led to dramatic improvement in a 25-year-old patient affected with severe inflammatory NS resistant to other treatments.⁹⁰ Infliximab infusions were given at a dose of 5 mg/kg at weeks 0, 2 and 6, and continued every other month for two years. Clinical improvement correlated with a reduction of TSLP levels in the skin and the serum, which could be used as a marker of disease activity. Although this first trial is promising, long-term efficacy and safety should be tested in a controlled study in a larger group of NS patients. In this setting, a specific treatment for NS is still missing. Certainly, knowledge of the NS pathomechanism has set up the basis for the development of novel therapeutic interventions which are constantly developing. As an example, since the investigations carried out in mouse models and NS patients have pointed to a KLK5 driven biological cascade as major pathogenetic event in NS, small molecules able to inhibit KLK5, or other serine proteases acting in the same pathway such as KLK7 or matriptase, could be ben-

eficial. In fact, a first multicenter clinical trial using topical application of a small KLK7 inhibitor is currently being performed in NS patients. Of course, determination of the benefit/risk ratio for this approach will still require some time. In the meantime the evaluation of the potential of a replacement peptide therapy using selected LEKTI fragments with physiological relevance and an appropriate delivery system, is underway. In parallel, gene addition has also been proposed. Actually, following proof-of-principle experiments⁹¹⁻⁹³ showing that production and release of LEKTI protein from even a small patch of skin exert a beneficial effect over a wide area, a phase I clinical trial has been proposed for treatment of patients with NS (clinical trials gov. NCT01545323). This study, which is not yet open for participant recruitment, aims at evaluating the safety and efficacy of grafting of autologous epidermal sheets generated from genetically modified skin stem cells. In conclusion, in the light of the spectacular progress made in the understanding of the NS pathogenetic mechanisms and LEKTI function/s, there is no doubt that the next few years will see important breakthroughs for therapeutic intervention for NS patients.

LEKTI and AD

Besides the well proven direct cause-effect relationship between LEKTI deficiency and NS, several genetic association studies suggested a contribution of LEKTI to the pathogenesis of AD, a frequent and chronic-relapsing inflammatory skin disease caused by complex interactions between genetics and environmental factors.⁹⁴ Specifically, an early candidate gene approach found an association between the non-synonymous and frequent c.1258G>A (p.E420K) polymorphism in the *SPINK5* gene and AD in two British cohorts.⁹⁵ Lengthily questioned, because of the contradictory results of several other studies,^{96, 97} this genetic association has very recently been functionally linked to the LEKTI biological activity.⁹⁸ Specifically, by *in vitro* and *in vivo* studies, Fortugno and co-workers have proved that the presence of a lysine at LEKTI 420 residue impacts on its proteolytic activation process and causes a slight but still relevant imbalance of the LEKTI inhibitory fragments.⁹⁸ Recalling in a much milder form the skin of NS patients, the 420KK individuals exhibit a little to moderate increase of KLK5, KLK7 and ELA2 activi-

ties in the epidermis as well as a slight reduction of DSG1, and accelerated pro-FLG proteolysis. In line with the emerging view that skin barrier abnormalities could represent primary events in AD pathogenesis,⁹⁹ it is conceivable to assume that the changes determined by the presence of residue 420K within the LEKTI sequence contribute to a subtle skin barrier permeability alteration. However, is also possible that the LEKTI altered proteolysis affects its inhibition capacities towards external proteases derived from allergens and *S. aureus*, as well as lipid maturation process and PAR2 mediated inflammation, all features which are relevant to AD pathogenesis.

As previously discussed, unrestrained KLK5 activity plays a major role in allergic manifestations in NS through an intrinsic KLK5-PAR2-TSLP pro-allergic pathway.^{62, 63} Consistent with KLK5 overactivity associated with the expression of LEKTI 420K variant, a weak but still detectable increase in TSLP expression was also observed in normal epidermis of individuals with a 420KK genotype.⁹⁸ While TSLP overexpression is a major characteristic of lesional atopic skin,⁶⁴⁻⁶⁶ the extent to which the 420K associated LEKTI abnormal processing favors the development of AD remains to be determined. In fact, considering the high frequency of the E420K variation and the weak genetic association with AD reported,⁹⁵⁻⁹⁷ it is possible that other determinants compensate for the effects of LEKTI abnormal proteolytic processing. In line with the multifactorial inheritance of the disease, this scenario is further corroborated by the fact that, in spite of the unquestioned recognition of *FLG* loss of function alleles as strong predisposing factors for AD, only ~42% of *FLG* null mutation heterozygous carriers becomes affected.¹⁰⁰ Additional studies aimed at investigating a potential synergic effect between LEKTI 420K and *FLG* mutations are certainly required. Anyhow, the new insights arising from the LEKTI functioning studies anticipate the restoration of the epidermal permeability barrier by re-establishment of LEKTI inhibitory activity as a feasible and promising therapeutic approach also for AD.

Riassunto

La sindrome di Netherton e la sua proteina deficitaria, la poliedrica LEKTI

La sindrome di Netherton (SN, OMIM 256500) è una rara malattia ad ereditarietà autosomica recessiva caratte-

rizzata da ittiosi congenita, una peculiare anomalia del fusto del capello, denominata tricoressi invaginata, e sintomi di atopia. A causa delle gravi complicanze che frequentemente si presentano in età neonatale, la prognosi della SN può essere infausta, in particolare nel primo anno di vita. La SN è determinata da mutazioni che portano alla formazione di codoni di stop nel gene *SPINK5* e alla conseguente mancanza di espressione nella cute e in tutti gli epitelii stratificati della proteina da esso codificata, LEKTI. In seguito all'identificazione del gene e della proteina che causano la SN sono stati sviluppati strumenti diagnostici specifici risolvendo in tal modo il difficile tema della diagnosi differenziale della SN rispetto ad altre forme di ittiosi congenite, le cui manifestazioni cliniche possono essere estremamente simili, nonché rispetto alle forme più severe a insorgenza precoce di dermatite atopica o psoriasi. Un notevole impegno è stato altresì dedicato alla comprensione dei meccanismi patogenetici della SN. Ciononostante, la gestione degli individui affetti da SN ancora rappresenta un difficile compito per la mancanza di trattamenti specifici ed i numerosi bisogni insoddisfatti. In questo articolo viene esaminato lo stato dell'arte delle conoscenze sulla SN con particolare enfasi sui progressi realizzati per lo sviluppo di terapie innovative specifiche per questa patologia.

PAROLE CHIAVE: Sindrome di Netherton - *SPINK5* - Serin proteasi - Infiammazione.

References

1. Comel M. Ichthyosis linearis circumflexa. *Dermatologica* 1949;98:133-6.
2. Netherton EW. A unique case of trichorrhoeis nodosa: bamboo hairs. *AMA Arch Derm* 1958;78:483-7.
3. Ong C, Harper J. Netherton's syndrome. In: Harper J, Orange A, Prose N, editors. *Textbook of Pediatric Dermatology*. Turin: Blackwell; 2006. p. 1359-66.
4. Traupe H. The Comel-Netherton syndrome. In: Traupe H, editor. *The Ichthyoses. A Guide to Clinical Diagnosis, Genetic Counseling and Therapy*. Berlin: Springer-Verlag; 1989. p. 168-78.
5. Pruszkowski A, Bodemer C, Fraitag S, Teillac-Hamel D, Amoric JC, de Prost Y. Neonatal and infantile erythrodermas: a retrospective study of 51 patients. *Arch Dermatol* 2000;136:875-80.
6. Magert HJ, Standker L, Kreutzmann P, Zucht HD, Reinecke M, Sommerhoff CP *et al*. LEKTI, a novel 15-domain type of human serine proteinase inhibitor. *J Biol Chem* 1999;274:21499-502.
7. Chavanas S, Bodemer C, Rochat A, Hamel-Teillac D, Ali M, Irvine AD *et al*. Mutations in *SPINK5*, encoding a serine protease inhibitor, cause Netherton syndrome. *Nat Genet* 2000;25:141-2.
8. Bitoun E, Micheloni A, Lamant L, Bonnart C, Tartaglia-Polcini A, Cobbold C *et al*. LEKTI proteolytic processing in human primary keratinocytes, tissue distribution and defective expression in Netherton syndrome. *Hum Mol Genet* 2003;12:2417-30.
9. Yang T, Liang D, Koch PJ, Hohl D, Kheradmand F, Overbeek PA. Epidermal detachment, desmosomal dissociation, and destabilization of corneodesmosin in *Spink5*^{-/-} mice. *Genes Dev* 2004;18:2354-58.
10. Descargues P, Deraison C, Bonnart C, Kreft M, Kishibe M, Ishida-Yamamoto A *et al*. *Spink5*-deficient mice mimic Netherton syndrome through degradation of desmoglein 1 by epidermal protease hyperactivity. *Nat Genet* 2005;37:56-65.
11. Hewett DR, Simons AL, Mangan NE, Jolin HE, Green SM, Fallon PG. Lethal, neonatal ichthyosis with increased proteolytic processing of filaggrin in a mouse model of Netherton syndrome. *Hum Mol Genet* 2005;14:335-46.
12. Descargues P, Deraison C, Prost C, Fraitag S, Mazereeuw-Hautier J, D'Alessio M *et al*. Corneodesmosomal cadherins are preferential targets of stratum corneum trypsin- and chymotrypsin-like hyperactivity in Netherton syndrome. *J Invest Dermatol* 2006;126:1622-32.
13. Hausser I, Anton-Lamprecht I. Severe congenital generalized exfoliative erythroderma in newborns and infants: a possible sign of Netherton syndrome. *Pediatr Dermatol* 1996;13:183-99.
14. Powell J. Increasing the likelihood of early diagnosis of Netherton syndrome by simple examination of eyebrow hairs. *Arch Dermatol* 2000;136:423-4.
15. Neri I, Balestri R, Starace M, Bardazzi F, Atrizi A. Videodermoscopy of eyelashes in Netherton syndrome. *J Eur Acad Dermatol Venereol* 2011;25:1360-1.
16. Rakowska A, Kowalska-Oledzka E, Slowinska M, Rosinska D, Rudnicka L. Hair shaft videodermoscopy in Netherton syndrome. *Pediatr Dermatol* 2009;26:320-2.
17. de Berker DA, Paige DG, Ferguson DJ, Dawber RP. Golf tee hairs in Netherton disease. *Pediatr Dermatol* 1995;12:7-11.
18. Goujon E, Beer F, Fraitag S, Hovnanian A, Vabres P. 'Matchstick' eyebrow hairs: a dermoscopic clue to the diagnosis of Netherton syndrome. *J Eur Acad Dermatol Venereol* 2010;24:740-1.
19. Sun JD, Linden KG. Netherton syndrome: a case report and review of the literature. *Int J Dermatol* 2006;45:693-7.
20. Judge MR, Morgan G, Harper JJ. A clinical and immunological study of Netherton's syndrome. *Br J Dermatol* 1994;131:615-21.
21. Giroux JD, Sizun J, Gardach C, Awad H, Guillois B, Alix D. Severe hypernatremic dehydration disclosing Netherton syndrome in the neonatal period. *Arch Fr Pediatr* 1993;50:585-8.
22. Stoll C, Alembik Y, Tchomakov D, Messer J, Heid E, Boehm N *et al*. Severe hypernatremic dehydration in an infant with Netherton syndrome. *Genet Couns* 2001;12:237-43.
23. Diociaiuti A, Castiglia D, Fortugno P, Bartuli A, Pascucci M, Zamburino G *et al*. Lethal Netherton syndrome due to homozygous p. Arg371X mutation in *SPINK5*. *Pediatric Dermatology* [In press].
24. Beljan G, Traupe H, Metz D, Sunderkotter C. Comel-Netherton syndrome with bacterial superinfection. *Hautarzt* 2003;54:1198-202.
25. Arslanagic N, Arslanagic R. Netherton syndrome with recurrent herpes of facial skin. *Med Arh* 2002;56:221-4.
26. Chao SC, Richard G, Lee JY. Netherton syndrome: report of two Taiwanese siblings with staphylococcal scaled skin syndrome and mutation of *SPINK5*. *Br J Dermatol* 2005;152:159-65.
27. Garty BZ, Nimri R. Hypothyroidism in Netherton syndrome. *Pediatr Dermatol* 2008;25:134-5.
28. Soreide K, Soiland H, Korner H, Haga H, Soreide JA. Acute pancreatitis in a young girl with the Netherton syndrome. *J Pediatr Surg* 2005;40:e69-e72.
29. Pohl M, Zimmerhackl LB, Hausser I, Ludwig H, Hildebrandt F, Gordjani N *et al*. Acute bilateral renal vein thrombosis complicating Netherton syndrome. *Eur J Pediatr* 1998;157:157-60.
30. Andre E, Till M, Descargues P, Cordier MP, Fouilloux A, Haftek M *et al*. Netherton syndrome: a type of infantile erythroderma with failure to thrive, immune deficiency, rickets. Report of 3 cases. *Arch Pediatr* 2005;12:1364-67.
31. Macknet CA, Morkos A, Job L, Garberoglio MC, Clark RD, Macknet KD Jr *et al*. An infant with Netherton syndrome and persistent pulmonary hypertension requiring extracorporeal membrane oxygenation. *Pediatr Dermatol* 2008;25:368-72.
32. Hintner H, Jaschke E, Fritsch P. Netherton syndrome: weakened immunity, generalized verrucosis and carcinogenesis. *Hautarzt* 1980;31:428-32.
33. Kubler HC, Kuhn W, Rummel HH, Kaufmann I, Kaufmann M.

- Development of cancer (vulvar cancer) in the Netherton syndrome (ichthyosis, hair anomalies, atopic diathesis). *Geburtshilfe Frauenheilkd* 1987;47:742-4.
34. Saghari S, Woolery-Lloyd H, Nouri K. Squamous cell carcinoma in a patient with Netherton's syndrome. *Int J Dermatol* 2002;41:415-6.
 35. Krasagakis K, Ioannidou DJ, Stephanidou M, Manios A, Panayiotides JG, Tosca AD. Early development of multiple epithelial neoplasms in Netherton syndrome. *Dermatology* 2003;207:182-4.
 36. Li AL, Walsh S, McKay DR. Surgical management of a giant condyloma of Buschke-Lowenstein in a patient with Netherton syndrome using the pedicle anterolateral thigh flap – a case report. *J Plast Reconstr Aesthet Surg* 2011;64:1533-6.
 37. Leclerc-Mercier S, Bodemer C, Bourdon-Lanoy E, Larousserie F, Hovnanian A, Brousse N, *et al.* Early skin biopsy is helpful for the diagnosis and management of neonatal and infantile erythrodermas. *J Cutan Pathol* 2009;37:249-55.
 38. Sprecher E, Chavanas S, DiGiovanna JJ, Amin S, Nielsen K, Prendiville JS *et al.* The spectrum of pathogenic mutations in *SPINK5* in 19 families with Netherton syndrome: implications for mutation detection and first case of prenatal diagnosis. *J Invest Dermatol* 2001;117:179-87.
 39. Bitoun E, Chavanas S, Irvine AD, Lonie L, Bodemer C, Paradisi M *et al.* Netherton syndrome: disease expression and spectrum of *SPINK5* mutations in 21 families. *J Invest Dermatol* 2002;118:352-61.
 40. Bitoun E, Bodemer C, Amiel J, de Prost Y, Stoll C, Calvas P *et al.* Prenatal diagnosis of a lethal form of Netherton syndrome by *SPINK5* mutation analysis. *Prenat Diagn* 2002;22:121-6.
 41. Sprecher E, Tesfaye-Kedjela A, Ratajczak P, Bergman R, Richard G. Deleterious mutations in *SPINK5* in a patient with congenital ichthyosiform erythroderma: molecular testing as a helpful diagnostic tool for Netherton syndrome. *Clin Exp Dermatol* 2004;29:513-7.
 42. Raghunath M, Tontsidou L, Oji V, Aufenvenne K, Schürmeyer-Horst F, Jayakumar A *et al.* *SPINK5* and Netherton syndrome: novel mutations, demonstration of missing LEKTI, and differential expression of transglutaminases. *J Invest Dermatol* 2004;123:474-83.
 43. Mizuno Y, Suga Y, Muramatsu S, Hasegawa T, Shimizu T, Ogawa H. A Japanese infant with localized ichthyosis linearis circumflexa on the palms and soles harbouring a compound heterozygous mutation in the *SPINK5* gene. *Br J Dermatol* 2005;153:661-3.
 44. Mizuno Y, Suga Y, Haruna K, Muramatsu S, Hasegawa T, Kohroh K *et al.* A case of a Japanese neonate with congenital ichthyosiform erythroderma diagnosed as Netherton syndrome. *Clin Exp Dermatol* 2006;31:677-80.
 45. Shimomura Y, Sato N, Kariya N, Takatsuka S, Ito M. Netherton syndrome in two Japanese siblings with a novel mutation in the *SPINK5* gene: immunohistochemical studies of LEKTI and other epidermal molecules. *Br J Dermatol* 2005;153:1026-30.
 46. Lin SP, Huang SY, Tu ME, Wu YH, Lin CY, Lin HY *et al.* Netherton syndrome: mutation analysis of two Taiwanese families. *Arch Dermatol Res* 2007;299:145-50.
 47. Lacroix M, Lacaze-Buzy L, Furio L, Tron E, Valari M, Van der Wier G *et al.* Clinical expression and new *SPINK5* splicing defects in Netherton syndrome: unmasking a frequent founder synonymous mutation and unconventional intronic mutations. *J Invest Dermatol* 2012;132:575-82.
 48. Chao SC, Tsai YM, Lee JY. A compound heterozygous mutation of the *SPINK5* gene in a Taiwanese boy with Netherton syndrome. *J Formos Med Assoc* 2003;102:418-23.
 49. Fong K, Akdeniz S, Isi H, Taskesen M, McGrath JA, Lai-Cheong JE. New homozygous *SPINK5* mutation, pGln333X, in a Turkish pedigree with Netherton syndrome. *Clin Exp Dermatol* 2010;36:412-5.
 50. Alpigiani MG, Salvati P, Schiaffino MC, Occella C, Castiglia D, Covaciu C *et al.* A new *SPINK5* mutation in a patient with Netherton syndrome: a case report. *Pediatr Dermatol* 2012;29:521-2.
 51. Di WL, Hennekam RC, Callard RE, Harper JJ. A heterozygous null mutation combined with the G1258A polymorphism of *SPINK5* causes impaired LEKTI function and abnormal expression of skin barrier proteins. *Br J Dermatol* 2009;161:404-12.
 52. Hachem JP, Wagberg F, Schmuth M, Crumrine D, Lissens W, Jayakumar A *et al.* Serine protease activity and residual LEKTI expression determine phenotype in Netherton syndrome. *J Invest Dermatol* 2006;126:1609-21.
 53. Komatsu N, Saijoh K, Jayakumar A, Clayman GL, Tohyama M, Suga Y *et al.* Correlation between *SPINK5* gene mutations and clinical manifestations in Netherton syndrome patients. *J Invest Dermatol* 2008;128:1148-59.
 54. Tüysüz B, Ojalvo D, Mat C, Zambruno G, Covaciu C, Castiglia D *et al.* A new *SPINK5* donor splice site mutation in siblings with Netherton syndrome. *Acta Derm Venereol* 2010;90:95-6.
 55. Renner ED, Hartl D, Rylaarsdam S, Young ML, Monaco-Shawver L, Kleiner G *et al.* Comel-Netherton syndrome defined as primary immunodeficiency. *J Allergy Clin Immunol* 2009;124:536-43.
 56. Kilic G, Guler N, Ones U, Tamay Z, Guzel P. Netherton syndrome: report of identical twins presenting with severe atopic dermatitis. *Eur J Pediatr* 2006;165:594-7.
 57. Zhao Y, Ma ZH, Yang Y, Yang SX, Wu LS, Ding BL *et al.* *SPINK5* gene mutation and decreased LEKTI activity in three Chinese patients with Netherton's syndrome. *Derm Clin Exp Dermatol* 2007;32:564-7.
 58. Fortugno P, Grosso F, Zambruno G, Pastore S, Faletra F, Castiglia D. A synonymous mutation in *SPINK5* exon 11 causes Netherton syndrome by altering exonic splicing regulatory elements. *J Hum Genet* 2012;57:311-5.
 59. Caubet C, Jonca N, Brattsand M, Guerrin M, Bernard D, Schmidt R *et al.* Degradation of corneodesmosome proteins by two serine proteases of the kallikrein family, SCTE/KLK5/hK5 and SCCE/KLK7/hK7. *J Invest Dermatol* 2004;122:1235-44.
 60. Borgoño CA, Michael IP, Komatsu N, Jayakumar A, Kapadia R, Clayman GL *et al.* A potential role for multiple tissue kallikrein serine proteases in epidermal desquamation. *J Biol Chem* 2007;282:3640-52.
 61. Bonnart C, Deraison C, Lacroix M, Uchida Y, Besson C, Robin A *et al.* Elastase 2 is expressed in human and mouse epidermis and impairs skin barrier function in Netherton syndrome through filaggrin and lipid misprocessing. *J Clin Invest* 2010;120:871-82.
 62. Briot A, Deraison C, Lacroix M, Bonnart C, Robin A, Besson C *et al.* Kallikrein 5 induces atopic dermatitis-like lesions through PAR2-mediated thymic stromal lymphopoietin expression in Netherton syndrome. *J Exp Med* 2009;206:1135-47.
 63. Briot A, Lacroix M, Robin A, Steinhoff M, Deraison C, Hovnanian A. Par2 inactivation inhibits early production of TSLP, but not cutaneous inflammation, in Netherton syndrome adult mouse model. *J Invest Dermatol* 2010;130:2736-42.
 64. Ziegler SF, Artis D. Sensing the outside world: TSLP regulates barrier immunity. *Nat Immunol* 2010;11:289-93.
 65. Liu YJ. Thymic stromal lymphopoietin: master switch for allergic inflammation. *J Exp Med* 2006;203:269-73.
 66. Soumelis V, Reche PA, Kanzler H, Yuan W, Edward G, Homey B *et al.* Human epithelial cells trigger dendritic cell mediated allergic inflammation by producing TSLP. *Nat Immunol* 2002;3:673-80.
 67. Yamasaki K, Schaubert J, Coda A, Lin H, Dorschner RA, Schechter NM *et al.* Kallikrein-mediated proteolysis regulates the antimicrobial effects of cathelicidins in skin. *FASEB J* 2006;20:2068-80.
 68. Ny A, Egelrud T. Epidermal hyperproliferation and decreased skin barrier function in mice overexpressing stratum corneum chymotryptic enzyme. *Acta Derm Venereol* 2004;84:18-22.
 69. Hansson L, Backman A, Ny A, Edlund M, Ekholm E, Ekstrand Hammarström B *et al.* Epidermal overexpression of stratum cor-

- neum chymotryptic enzyme in mice: a model for chronic itchy dermatitis. *J Invest Dermatol* 2002;118:444-9.
70. Deraison C, Bonnart C, Robin A, Briot A, Besson C, Segre J *et al*. KLK5 transgenic mice reproduce NS phenotype. *J Invest Dermatol* 2007;127:S54.
 71. Sales KU, Masedunskas A, Bey AL, Rasmussen AN, Weigert R, List K *et al*. Matrilysin initiates activation of epidermal pro-kal-likrein and disease onset in a mouse model of Netherton syndrome. *Nat Genet* 2010;42:676-83.
 72. Elias PM, Williams ML, Crumrine D, Schuth M. Inherited disorders of accelerated desquamation. Netherton syndrome. In: Itin P, editors. *Ichthyoses. Clinical, Biochemical, Pathogenic and Diagnostic Assessment*. Basel, Switzerland: Karger; 2010. p. 89-97.
 73. Bennett K, Heywood W, Di WL, Harper J, Clayman GL, Jayakumar A *et al*. The identification of a new role for LEKTI in the skin: the use of protein 'bait' arrays to detect defective trafficking of dermcidin in the skin of patients with Netherton syndrome. *J Proteom* 2012;75:3925-37.
 74. Rieg S, Steffen H, Seeber S, Humeny A, Kalbacher H, Dietz K *et al*. Deficiency of dermcidin-derived antimicrobial peptides in sweat of patients with atopic dermatitis correlates with an impaired innate defense of human skin in vivo. *J Immunol* 2005;174:8003-10.
 75. Ovaere P, Lippens S, Vandenabeele P, Declercq W. The emerging roles of serine protease cascades in the epidermis. *Trends Biochem Sci* 2009;34:453-63.
 76. Tartaglia-Polcini A, Bonnart C, Micheloni A, Cianfarani F, André A, Zambruno G *et al*. *SPINK5*, the defective gene in netherton syndrome, encodes multiple LEKTI isoforms derived from alternative pre-mRNA processing. *J Invest Dermatol* 2006;126:315-24.
 77. Fortugno P, Bresciani A, Paolini C, Pazzagli C, El Hachem M, D'Alessio M *et al*. Proteolytic activation cascade of the Netherton syndrome-defective protein, LEKTI, in the epidermis: implications for skin homeostasis. *J Invest Dermatol* 2011;131:2223-32.
 78. Ishida-Yamamoto A, Deraison C, Bonnart C, Bitoun E, Robinson R, O'Brien TJ *et al*. LEKTI is localized in lamellar granules, separated from KLK5 and KLK7, and is secreted in the extracellular spaces of the superficial stratum granulosum. *J Invest Dermatol* 2005;124:360-6.
 79. Deraison C, Bonnart C, Lopez F, Besson C, Robinson R, Jayakumar A. LEKTI fragments specifically inhibit KLK5, KLK7, and KLK14 and control desquamation through a pH-dependent interaction. *Mol Biol Cell* 2007; 18 :3607-19.
 80. Allen A, Siegfried E, Silverman R, Williams ML, Elias PM, Szabo SK *et al*. Significant absorption of topical tacrolimus in 3 patients with Netherton syndrome. *Arch Dermatol* 2001;137:747-50.
 81. Shah KN, Yan AC. Low but detectable serum levels of tacrolimus seen with the use of very dilute, extemporaneously compounded formulations of tacrolimus ointment in the treatment of patients with Netherton syndrome. *Arch Dermatol* 2006;142:1362-3.
 82. Saif GB, Al-Khenaizan S. Netherton syndrome: successful use of topical tacrolimus and pimecrolimus in four siblings. *Int J Dermatol* 2007;46:290-4.
 83. Oji V, Beljan G, Beier K, Traupe H, Luger TA. Topical pimecrolimus: a novel therapeutic option for Netherton syndrome. *Br J Dermatol* 2005;153:1067-8.
 84. Henno A, Choffray A, De La Brassinne M. Improvement of Netherton syndrome associated erythroderma in two adult sisters through use of topical pimecrolimus. *Ann Dermatol Venereol* 2006;133:71-2.
 85. Yan AC, Honig PJ, Ming ME, Weber J, Shah KN. The safety and efficacy of pimecrolimus, 1%, cream for the treatment of Netherton syndrome: results from an exploratory study. *Arch Dermatol* 2010;146:57-62.
 86. Halverstam CP, Vachharajani A, Mallory SB. Cushing syndrome from percutaneous absorption of 1% hydrocortisone ointment in Netherton syndrome. *Pediatr Dermatol* 2007;24:42-5.
 87. Renner ED, Hartl D, Rylaarsdam S, Young ML, Monaco-Shawver L, Kleiner G *et al*. Comel-Netherton syndrome defined as primary immunodeficiency. *J Allergy Clin Immunol* 2009;124:536-43.
 88. Maatouk I, Moutran R, Tomb R. Narrowband ultraviolet B phototherapy associated with improvement in Netherton syndrome. *Clin Exp Dermatol* 2012;37:364-6.
 89. Kaminska EC, Ortel B, Sharma V, Stein S. Narrowband UVB phototherapy as a novel treatment for Netherton syndrome. *Photoderma Photoimmunol Photomed* 2012;28:162-4.
 90. Fontao L, Laffitte E, Briot A, Kaya G, Roux-Lombard P, Fraitag S *et al*. Infliximab infusions for Netherton syndrome: sustained clinical improvement correlates with a reduction of thymic stromal lymphopoietin levels in the skin. *J Invest Dermatol* 2011;131:1947-50.
 91. Di WL, Larcher F, Semenova E, Talbot GE, Harper JI, Del Rio M *et al*. Ex vivo gene therapy restores LEKTI activity and corrects the architecture of Netherton syndrome-derived skin grafts. *Mol Ther* 2011;19:408-16.
 92. Roedl D, Oji V, Buters JT, Behrendt H, Braun-Falco M. rAAV2-mediated restoration of LEKTI in LEKTI-deficient cells from Netherton patients. *J Dermatol Sci* 2011;61:194-8.
 93. Di WL, Semenova E, Larcher F, Del Rio M, Harper JI, Thrasher AJ *et al*. Human involucrin promoter mediates repression resistant and compartment specific LEKTI expression. *Hum Gene Ther* 2012;23:83-90.
 94. Leung Y, Bibier T. Atopic dermatitis. *Lancet* 2003;361:151-60.
 95. Walley AJ, Chavanas S, Moffatt MF, Esnouf RM, Ubhi B, Lawrence R *et al*. Gene polymorphism in Netherton and common atopic disease. *Nat Genet* 2001;29:175-8.
 96. Fölster-Holst R, Stoll M, Koch WA, Hampe J, Christophers E, Schreiber S. Lack of association of *SPINK5* polymorphisms with nonsyndromic atopic dermatitis in the population of Northern Germany. *Br J Dermatol* 2005;152:1365-7.
 97. Jongepier H, Koppelman GH, Nolte IM, Bruinenberg M, Bleecker ER, Meyers DA *et al*. Polymorphisms in *SPINK5* are not associated with asthma in a Dutch population. *J Allergy Clin Immunol* 2005;115:486-92.
 98. Fortugno P, Furio L, Teson M, Berretti M, El Hachem M, Zambruno G *et al*. The 420K LEKTI variant alters LEKTI proteolytic activation and results in protease deregulation: implications for atopic dermatitis. *Hum Mol Genet* 2012;21:4187-200.
 99. Cork MJ, Danby SG, Vasilopoulos Y, Hadgraft J, Lane ME, Moustafa M *et al*. Epidermal barrier dysfunction in atopic dermatitis. *J Invest Dermatol* 2009;129:1892-908.
 100. Henderson J, Northstone K, Lee SP, Liao H, Zhao Y, Pembrey M *et al*. The burden of disease associated with filaggrin mutations: a population-based, longitudinal birth cohort study. *J Allergy Clin Immunol* 2008;121:872.e9-877.e9.