Skin Barrier Function and mRNA Expression Profiles in Patients with Atopic Dermatitis, Ichthyosis Vulgaris, and X-linked Recessive Ichthyosis:

Aetiopathogenic Differences and the Impact of Moisturizing Treatment

TORBORG STURESDOTTER HOPPE
Atopic dermatitis (AD), ichthyosis vulgaris (IV), and X-linked recessive ichthyosis (XLRI) are characterized by dry skin and impaired skin barrier. AD and IV are related to loss-of-function mutations in FLG (encoding filaggrin), whereas XLRI is caused by deletions or inactivating mutations in the steroid sulphatase gene (STS). Patients regularly use moisturizing creams, but little is known about the creams’ effects on the skin barrier.

The present work combines objective scorings, non-invasive techniques, and molecular analyses of skin biopsies to characterize the skin in 57 patients with AD, IV, or XLRI, and in 14 healthy controls. Patients were classified according to their FLG and STS mutation status: AD with FLG+/+ (n = 14), AD with FLG+/− (n = 14), AD/IV with FLG−/− (n = 15), and XLRI with STS− (n = 14), as well as one man with a novel point mutation. Assessments were conducted at baseline and after four weeks of treatment with three different moisturizers applied to volar forearm skin.

At baseline, dryness scoring and non-invasive assessments verified impaired skin barrier function in all patients. In patients with AD/IV, microarray analysis identified 300–3000 up- or downregulated mRNA transcripts involved in signalling pathways important for inflammation and barrier repair. The skin phenotype and number of altered transcripts were correlated with the FLG mutation status, with FLG−/− patients displaying the highest transepidermal water loss (TEWL) and the most altered transcript levels. In contrast, despite an equally dysfunctional skin barrier, only limited changes in mRNA transcripts occurred in XLRI patients. Treatment with moisturizers improved skin dryness similarly in all groups, but TEWL behaved differently: it decreased slightly in the AD/IV group and increased in the XLRI group, especially after urea treatment. Only minute effects on skin pH and mRNA expression were observed.

In conclusion, FLG mutations elicit pro-inflammatory mechanisms probably aimed at restoring barrier competence. This does not occur in patients with XLRI, presumably because STS deficiency automatically increases the barrier thickness. Moisturizing treatment improves skin dryness in patients with AD, IV, or XLRI, but does not seem to normalize the altered epidermal gene expression profile in AD/IV patients.

Keywords: atopic dermatitis, ichthyosis vulgaris, X-linked recessive ichthyosis, skin barrier function, moisturizers, transepidermal water loss, gene expression
To my family

“Yesterday is history, tomorrow is a mystery, but today is a gift. That's why we call it the present.”

A.A. Milne “Winnie the Pooh”
List of Papers

This thesis is based on the following papers, which are referred to in the text by their Roman numerals:


III: Hoppe T, Winge MCG, Bradley M, Nordenskjöld M, Vahlquist A, Törmä H, Berne B. Moisturizing treatment of patients with atopic dermatitis and ichthyosis vulgaris improves dry skin but has a modest effect on gene expression regardless of FLG genotype. *Submitted*.


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<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Full Form</th>
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<tbody>
<tr>
<td>AD</td>
<td>Atopic dermatitis</td>
</tr>
<tr>
<td>CE</td>
<td>Cornified envelope</td>
</tr>
<tr>
<td>CSO₄</td>
<td>Cholesterol sulphate</td>
</tr>
<tr>
<td>FASN</td>
<td>Fatty acid synthase</td>
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<tr>
<td>FLG</td>
<td>Filaggrin</td>
</tr>
<tr>
<td>GBA</td>
<td>β-glucocerebrosidase</td>
</tr>
<tr>
<td>HMGCR</td>
<td>3-hydroxy-3-methylglutaryl-CoA reductase</td>
</tr>
<tr>
<td>IL</td>
<td>Interleukin</td>
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<tr>
<td>IV</td>
<td>Ichthyosis vulgaris</td>
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<tr>
<td>IVL</td>
<td>Involucrin</td>
</tr>
<tr>
<td>NMFs</td>
<td>Natural moisturizing factors</td>
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<tr>
<td>PPAR</td>
<td>Peroxisome proliferator-activated receptor</td>
</tr>
<tr>
<td>SC</td>
<td>Stratum corneum</td>
</tr>
<tr>
<td>SCORAD</td>
<td>Scoring atopic dermatitis index</td>
</tr>
<tr>
<td>SPTLC2</td>
<td>Serine-palmitoyl-CoA transferase 2</td>
</tr>
<tr>
<td>STS</td>
<td>Steroid sulphatase</td>
</tr>
<tr>
<td>TEWL</td>
<td>Transepidermal water loss</td>
</tr>
<tr>
<td>TGM</td>
<td>Transglutaminase</td>
</tr>
<tr>
<td>UCA</td>
<td>Trans-urocanic acid</td>
</tr>
<tr>
<td>XLRI</td>
<td>X-linked recessive ichthyosis</td>
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</table>
Introduction

Skin structure and function

The skin is the body’s largest organ, approaching 5 kg and 2 m² in a 70-kg individual. The primary function of the skin is to maintain an internal environment suitable for life on dry land. The skin protects us from dehydration, mechanical damage, UV radiation, and other potentially harmful factors. It is not, however, just a shield but an active organ playing an important role in the immune system, nervous system, in the metabolism of vitamin D, and in psychosocial communication.

The skin is divided into the epidermis, dermis, and subcutis (Fig. 1). Starting from the bottom, the subcutis is a layer of adipose tissue serving as an energy source as well as insulation. It allows the skin to move over underlying structures and gives our body its outer contour.

Above the subcutis is the dermis, which provides structural and nutritional support to the skin. The dermis is well vascularized and contains both sensory and motor nerve fibres as well as sebaceous glands and sweat glands. The dermis is in turn divided into two layers, the deeper reticular dermis and the more superficial papillary dermis. The reticular dermis consists of a thicker layer of connective tissue with larger blood vessels, bundles of collagen fibres arranged in layers parallel to the skin surface, and elastic fibres providing flexibility and strength. The papillary dermis is thinner, consisting of loose connective tissue with elastic fibres, collagen, and capillaries. Here we find fibroblasts and various cell types such as macrophages, mast cells, and lymphocytes. The dermis varies in thickness from 0.5 mm on the eyelids to 1.5–5.0 mm on the back.

The outermost layer of the skin is the epidermis, which is tightly connected to the dermis by the basal membrane zone. The dominant cell type in the epidermis is the keratinocytes, accounting for 90–95% of the cell mass. The rest consists of melanocytes, Langerhans’ cells, Merkel cells, and various immune system cells. The epidermis is 0.05–0.10 mm thick, except for the thicker skin of palms and soles, and is in turn divided into four layers, depending on the maturity of the keratinocytes: the stratum basale, stratum spinosum, stratum granulosum, and stratum corneum (Fig. 2). As the keratinocytes move from the stratum basale up to the stratum corneum, they mature from columnar cells with stem cell properties to flat anucleated corneocytes filled with keratin. During this process, known as cornification,
the plasma membrane is replaced by an envelope of cross-linked proteins and lipids, the cornified envelope (CE). For a review, see *Rook’s Textbook of Dermatology*.

*Fig. 1.* The various layers of the skin. Illustration by M Hoppe.
The skin barrier resides in the epidermis.

The stratum corneum (SC) is the most prominent element of “the skin barrier”. This top layer of the epidermis, only 10–20 µm thick, acts as a physical barrier that protects us from water loss (the inside–out barrier) and from harmful substances entering the body (the outside–in barrier). The SC also acts as a chemical/antimicrobial barrier, via its lipids, acids, hydrolytic enzymes, and antimicrobial peptides, and as an immunological barrier via the humoral and cellular components of the immune system.

The SC is often likened to a brick wall, with packed corneocytes acting as bricks and the intercellular lipids as mortar (Fig. 2). Specialized protein structures, corneodesmosomes, act as “rivets”, holding the corneocytes together (for a review, see Elias3). The production of this complex structure relies on the continuous proliferation and differentiation of keratinocytes. The cornification process involves many structural proteins and enzymes. An early component in CE formation is involucrin (IVL), providing a scaffold to which other proteins and lipids subsequently become attached. A key protein is filaggrin (FLG), which contributes by binding to and aggregating the keratin bundles and by flattening the filament network of the keratinocytes, resulting in the compressed corneocytes. FLG will be discussed in more detail later. Transglutaminase (TGM) 1, 3, and 5 participate by crosslinking proteins such as IVL, envoplakin, periplakin, loricrin, and small proline-like proteins under the cell surface to form the CE.
The intercellular space in the SC consists of three major classes of lipids: ceramides (45–50%), cholesterol (25%), and free fatty acids (10–15%). Approximately 5% are other lipids, predominantly cholesterol sulphate.

The lipids are synthesized within the keratinocytes during differentiation. They are then stored in lamellar bodies, small organelles originating from the Golgi apparatus, enriched in glycosylceramides, cholesterol, phospholipids, and various catabolic enzymes. At the transition from granular cell to corneocyte, the lamellar bodies fuse with the plasma membrane and secrete their contents into the intercellular space. The lipids are then modified and arranged into lamellar structures surrounding the corneocytes.

Ceramides are synthesized by serine palmitoyl-CoA transferase (SPT) and by the conversion of glucosylceramides and sphingomyelin to ceramides by β-glucocerebrosidase (GBA) and sphingomyelinase, respectively. There are several different forms of ceramides in human SC. One unique class of ceramides is ω-hydroxyacyl-sphingosine. This ceramide is hydrolyzed into ω-hydroxylceramide by lipoxygenases, and in turn is covalently attached to the CE by TGM-1. These covalently bound ceramides constitute the backbone of the arrangement of the intercellular lipid bilayer and are important for the integrity of the water barrier. Most of the cholesterol is
synthesized de novo in SC from acetate; to a lesser extent, cholesterol is also released from cholesterol sulphate. The third major lipid class, free fatty acids, is synthesized by the conversion of phospholipids by phospholipase A and acid lipases. Free fatty acids are also obtained directly from the diet via the blood.

Filaggrin

FLG is a key player in normal skin barrier function. FLG is initially synthesized as profilaggrin, a large protein rich in histidine and glutamine, stored in keratohyalin granules in the stratum granulosum. In the later stages of epidermal terminal differentiation, profilaggrin is dephosphorylated and cleaved into 10–12 filaggrin repeats in the SC. Filaggrin contributes to CE formation and is subsequently degraded into free amino acids. These amino acids, together with their derivates, trans-urocanic acid (UCA) and pyrrolidone carboxylic acid (PCA), are major components of natural moisturizing factors (NMFs), important for epidermal hydration. UCA also provides protection against UV radiation.

The importance of FLG for a protective and moisturized skin barrier is illustrated by the aetiology of two common skin disorders, atopic dermatitis (AD) and ichthyosis vulgaris (IV). Single, compound, or dual mutations in the gene coding for filaggrin (FLG) are major predisposing factors for AD, and dual loss-of-function mutations in the same gene cause IV. Several different FLG mutations have been identified, all leading to the formation of truncated, non-functional protein. The four most prevalent mutations in the European population are R501X, 2282del4, S3247X, and R2447X. However, mutations do not always result in skin symptoms, since 7–10% of the general European population are heterozygous carriers of these mutations.

Since breakdown products of filaggrin constitute some of the NMFs, carriers of loss-of-function mutations in FLG have reduced levels of NMFs in the SC. The quantity of NMFs corresponds to the FLG mutation status and to the number of FLG repeats. It has also been demonstrated that, when loss-of-function mutations are excluded, the odds of having AD decrease with each additional FLG repeat.

Skin hydration

The water content in SC is normally approximately 10–15%, but can vary depending on ambient conditions and the amounts of NMFs and lipids in the SC. The characteristics of the SC are markedly influenced by the water content. At a low water content, the skin surface becomes less flexible and tends to crack. The subjective feeling of dry skin is often a direct result of
reduced water content in the outermost layers of the SC, but can also reflect a deficiency of surface lipids.

**Skin pH**

The surface pH of healthy, undamaged skin is slightly acid, approximately 5.4, and increases to approximately 7 in the lower SC, forming a steep pH gradient across the SC. The exact mechanism behind this is still unknown, but several factors are thought to contribute, such as NMFs, lactic acid, free fatty acids, and amino acids secreted on the skin surface. A reduction in NMFs, as in filaggrin-deficient skin, could consequently lead to a less acidic pH, and this has also been demonstrated in studies of AD patients.

An acidic skin surface inhibits the growth of pathogenic microorganisms, and is thus among the skin’s protective properties. The pH gradient across the SC is also an important regulator of desquamation and the generation of intercellular lipids, as several enzymes are pH dependent. A more neutral or alkaline milieu leads to the inhibition of intercellular lipid synthesis and enhanced protease activity, resulting in the breakdown of corneodesmosomes. As a result, the skin barrier is perturbed.

**Non-invasive measurements of skin function**

**Transepidermal water loss**

The gold standard for assessing skin barrier function is to measure transepidermal water loss (TEWL) in g of water m⁻² hour⁻¹, using an evaporimeter, reflecting the physiological fact that a disturbed skin barrier permits more water to pass through. This is a well-known and validated tool for assessing the skin barrier function in healthy skin; however, it is not as thoroughly validated for diseased skin. Studies have demonstrated that TEWL is higher in people with skin conditions involving a disturbed skin barrier function, such as AD and ichthyosis, than in healthy controls.

It is important to realize that TEWL is a marker of the inside–out, but not the outside–in, skin barrier function. To study the latter, one must perform penetration studies. In most cases, however, these two processes seem to correlate quite well. TEWL measurement is non-invasive and easy to perform, but could be influenced by exogenous factors such as temperature and humidity in the examination room. This makes it important to minimize variations in the indoor climate in which the measurements are made. The measurement can also be influenced by sweating and by occlusive emollients, but is not influenced by age, sex, or time of day, and does not seem to be influenced by vasoconstriction or dilatation.
Measurement of skin hydration
In daily practice, skin dryness is readily evaluated by the investigator touching the skin (semi-objective) or by asking the patients themselves (subjective). The most common methods to assess SC hydration are based on measuring electrical capacitance or impedance as an indirect indicator of SC water content. These methods reflect the fact that electric impulses are transmitted differently through hydrated versus dry areas. Confocal Raman microspectroscopy is another option for determining skin hydration and the SC water gradient. This method is based on the inelastic light scattering of various molecules.

In general, SC hydration is correlated with TEWL values: normal hydration reflects normal TEWL, while a higher proportion of water is lost through a dry, flaky skin, for example, in disorders with impaired skin barrier function. In specific anatomic regions, such as the palmo-plantar areas, there can be discrepancies in the correlation between TEWL and SC hydration.

Skin conditions with impaired skin barrier function
Atopic dermatitis
AD is a common, chronic, relapsing inflammatory skin condition characterized by early onset of dry skin and eczema, affecting up to 25% of children and 1–3% of the adult population in Western countries. Studies have demonstrated that 20–50% of the European and Asian AD population display mutations in FLG, whereas a lower prevalence has been indicated in patients of African descent. Patients with AD and single or dual mutations in FLG tend to have a more severe disease, and an increased propensity to develop other atopic manifestations, than do patients without FLG mutations.

In addition to filaggrin dysfunction and other genetic factors, both environmental and individual trigger factors have been demonstrated to be important for AD development. A combination of inherited barrier abnormalities and acquired insults are thought to alter epidermal structure. This leads to an impaired inside–out barrier, seen as an increase in TEWL, and a disturbed outside–in barrier with an enhanced risk of allergen penetration and susceptibility to cutaneous infections. This predisposes to increased allergen presentation, in turn followed by immune activation with negative impact on skin barrier homeostasis. Changed enzymatic activity and changes in the gene expression pattern with induced inflammatory genes and repressed lipid metabolism genes have also been demonstrated.
Ichthyosis

Ichthyosis is a heterogeneous family of generalized, mostly genetic, skin disorders. The common denominator is dry and scaly skin. The severity can vary considerably between affected individuals with different kinds of ichthyosis.39 The most common forms of ichthyosis are ichthyosis vulgaris (IV) and X-linked recessive ichthyosis (XLRI).

Ichthyosis vulgaris

IV has a prevalence of at least 1:250. The characteristic phenotype of IV includes dry skin with fine scaling – most prominent over the arms, legs, and lower abdomen – palmar hyperlinearity, and keratosis pilaris. The symptoms often improve in a humid summer climate. IV is known to be associated with AD and other atopic manifestations such as asthma and hay fever.11,40 In 2006, Smith et al. demonstrated that IV is caused by loss-of-function mutations in FLG.11 It is considered to be an autosomal, semi-dominant condition.

X-linked recessive ichthyosis

XLRI affects 1:2000–1:6000 males; female carriers, with very few exceptions, do not manifest the disease. Patients with XLRI typically display persistent scaling of the skin with polygonal, regular dark scales. The limbs, trunk, neck, ears, and scalp are often involved, while the palms and soles are spared. The affected regions are often symmetrically distributed and more evident on the extensor aspects of the limbs, but can also involve the flexural sides. Extracutaneous signs are described involving corneal opacities, cryptorchidism, and a family history of delayed progression of parturition.41

XLRI is in most cases caused by deletions, in fewer cases by inactivating mutations, in the steroid sulfatase (STS) gene,41 leading to a lack of steroid sulfatase.42 Due to this enzyme deficiency, cholesterol sulphate (CSO₄) accumulates in several tissues, including the epidermis. As a result, the generation of cholesterol is reduced (see above). In addition, CSO₄ has an inhibitory effect on HMG CoA reductase (HMGCR), the rate-limiting enzyme in cholesterol synthesis.2 CSO₄ is also known to influence other biological processes; for example, it is a regulator of both epidermal differentiation and cornodesmosome degradation. Excess CSO₄ therefore leads to defect packing of the intercellular lipids, abnormal cornocyte retention, and a thickened SC.43,44 Patients with XLRI do not have an enhanced risk of eczema, but coexisting FLG mutations can have a modifying effect on the phenotype, resulting in more severe symptoms.35
Treatment

AD therapy is based on symptomatic treatment, with moisturizers as baseline therapy and anti-inflammatory treatment with topical corticosteroids or calcineurin inhibitors for the flare-ups. Individuals with more widespread disease can benefit from UV treatment. In a limited number of cases, oral immunosuppressive treatments, such as systemic corticosteroids or cyclosporine, can be indicated.46,47 In IV and XLRI, the most common treatments are moisturizers with or without a keratolytic additive. Systemic retinoids are only rarely indicated in these types of ichthyosis.48

Moisturizers

The key role of a moisturizer is to smoothen and rehydrate the skin. The most common topical formulations used for moisturizers are creams, but other formulations such as ointments, gels, pastes, or liquid preparations are also used. In its simplest form, a cream consists of two phases, usually oil and water, producing an oil-in-water (O/W) or water-in-oil (W/O) emulsion. To this emulsion other substances, such as humectants, emulsifiers, lipids, and antimicrobial preservatives, can be added to create various desired effects. The most commonly used humectants are urea, glycerine, and propylene glycol.49

Patients with AD and ichthyosis are often dependent on using moisturizers, sometimes several times a day. The lifetime moisturizer consumption for a person with ichthyosis has been estimated to be up to 1 tonne.50 Urea- and glycerol-containing moisturizers have produced beneficial effects in terms of disease severity scoring, fewer relapses in eczema, and decreased TEWL in patients with AD51,52 and IV.53

Despite this, little is known about the cellular and biochemical effects of moisturizing treatment. Previous studies of healthy subjects have found that treatment with various moisturizers has both beneficial and negative effects on the skin barrier function.54-56 Altered expression of genes important for the formation of skin barrier lipids and of genes involved in keratinocyte differentiation and desquamation has also been found.57,58 Knowledge is even more limited when it comes to the effects of moisturizers on an already impaired skin barrier.
Aims of the present work

The overall aim was to examine the skin barrier function in patients with AD, IV, or XLRI, i.e., in individuals with impaired skin barrier function due to different inherited defects.

The specific aims were:

– to investigate the functional and molecular alterations in the skin of patients with AD, IV, or XLRI compared with that of healthy individuals

– to examine whether functional and molecular alterations in the skin of patients with AD/IV are dependent on FLG mutation status

– to analyze the effects of moisturizing treatment in terms of functional and molecular alterations in the skin of patients with AD, IV, or XLRI, and to determine whether FLG or STS mutation status affects the response to moisturizing treatment

– to explore whether moisturizer composition influences treatment response
Materials and methods

The study was conducted according to the Declaration of Helsinki principles and was approved by the regional ethics committees of Uppsala University and the Karolinska Institute, and by the Swedish Medical Product Agency. Written informed consent and health declarations were obtained from all participants before inclusion in the study.

Patients and controls

Patients \((n = 57)\) diagnosed with AD, IV, or XLRI together with 14 healthy controls (volunteer subjects without past or present history of AD, dry skin, or other atopic manifestations) were identified at the dermatology departments of Uppsala University Hospital, Karolinska University Hospital Solna, and Sophiahemmet, Stockholm. A number of AD/IV patients were recruited from Swedish families with known \(FLG\) mutation status, as described previously.\(^{59}\) Exclusion criteria for both patients and healthy controls were pregnancy, other concomitant skin disease, and treatment within the previous four weeks with UV phototherapy, topical or systemic corticosteroids, immunosuppressants, or systemic retinoids.
Clinical examination and assessments

A dermatologist clinically examined patients and recorded their medical histories, using a standardized questionnaire, before inclusion in the study. AD was diagnosed according to the UK Working Party’s diagnostic criteria.60 Any other atopic manifestations, such as allergic asthma and allergic rhino-conjunctivitis, were assessed via the questionnaire.

Blood sampling and genotyping

Blood samples were taken from all patients. In all patients, FLG genotyping was performed using allelic discrimination for the most prevalent European FLG mutations, i.e., R501X, S3247X, and R2447X, as described in paper I. The STS gene in all male patients was subjected to multiplex ligation-dependent probe amplification (MLPA) analysis, as fully described in paper II. In healthy controls selected for oligonucleotide array analysis (see below), cDNA from skin biopsies was used for genotyping.

Patients were divided into four groups depending on FLG mutation status and the results of STS– genotyping: FLG+/+ with AD (n = 14), FLG+/- with AD (n = 14), FLG–/– with AD/IV (n = 15), and STS– / FLG+/+ with XLRI (n = 14). For details regarding ages, gender, and mutations, see Table 1.
Table 1. Description of the 57 patients and 14 healthy controls included in the study with respect to age, sex, mutation status, and clinical presentation.

<table>
<thead>
<tr>
<th>Patient group</th>
<th>Healthy controls (n = 14)</th>
<th>FLG+/+ (n = 14)</th>
<th>FLG+/- (n = 14)</th>
<th>FLG--/ (n = 15)</th>
<th>XLRI (n = 14)</th>
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<tbody>
<tr>
<td><strong>Age</strong></td>
<td></td>
<td></td>
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<tr>
<td>Mean (Range)</td>
<td>50 yrs (24–75)</td>
<td>54 yrs (28–71)</td>
<td>56 yrs (28–78)</td>
<td>59 yrs (44–75)</td>
<td>44 yrs (19–72)</td>
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<tr>
<td>Sex</td>
<td>6 females 8 males</td>
<td>9 females 5 males</td>
<td>11 females 3 males</td>
<td>7 females 8 males</td>
<td>Only males</td>
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<td>Mutations</td>
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<td>2282del4 (n = 13)</td>
<td>R501X* (n = 2)</td>
<td>Deletion (n = 13)</td>
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<td></td>
<td>2282del4* (n = 9)</td>
<td>S3247X* (n = 1)</td>
<td>2282del4/</td>
<td>Novel mutation (n = 1)</td>
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<td>S3247X (n = 2)</td>
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<td>AD/IV</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>IV (n = 8)**</td>
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</table>

* Homozygous mutations
** The IV group included patients with (n = 5) and without (n = 3) previous AD history.

**SCORAD, dryness score, TEWL, and pH**

The disease severity for AD was assessed using the scoring AD index (SCORAD).\textsuperscript{61} Total SCORAD assessment, of the whole body, was performed to characterize the overall severity of AD. Local SCORAD
assessment, addressing the same parameters as total SCORAD but on volar forearm skin only, was performed to evaluate local severity.

To assess the dryness of volar forearm skin, a four-point scale was used, based on the dryness evaluation included in SCORAD, i.e., none (0), mild (1), moderate (2), or severe (3); hereinafter, this is referred to as the dryness score.

TEWL was assessed using Tewameter TM300® and skin pH was measured using the Skin-pH-Meter® PH905 (both Courage + Khazaka electronic GmbH, Cologne, Germany). All assessments were repeated after the four-week treatment period with moisturizers. All parameters in this study were analyzed on volar forearm skin. This site is often used for analysis of TEWL and pH in clinical studies and for studying the skin penetration and effects of topical drugs, but is probably not representative of other parts of the body.

Biopsies and RNA extraction

Punch biopsies were taken from all patients and controls. One 3-mm punch biopsy was obtained from non-eczematous, non-scaly, volar forearm skin, after locally anesthetizing with lidocaine hydrochloride combined with adrenalin (Astra Zeneca, Södertälje, Sweden). This process was repeated in the patients, on both forearms, after the four-week treatment period with moisturizers. The biopsies were placed in RNA later® (Life Technologies, Stockholm, Sweden), dried, and subsequently frozen and stored at –70°C.

The skin biopsies were trimmed of all subcutaneous fat and as much of dermis as possible, placed in 1 mL of TRIzol® (Life Technologies), and subsequently homogenized using a Polytron homogenizer as described elsewhere. Total RNA was isolated and its concentration was determined by means of spectrophotometric analysis as previously described.

Analysis of mRNA expression using Affymetrix gene chip-probe arrays

After removing outliers in TEWL and pH values, four or five individuals were randomly selected from each of the five groups. Microarray gene expression, data processing, quality control, and statistical analysis were performed as described in detail in paper I. Genes of potential interest (i.e., those being > two-fold up- or downregulated) were analyzed using the Database for Annotation Visualization and Integrated Discovery (DAVID) functional annotation tool with Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis. Functional annotations were also carried out using Ingenuity pathway analysis software (Ingenuity Systems, Inc., Redwood City, CA). Up- and downregulated transcripts were analyzed for
their enrichment in human cytoband regions and gene ontology (GO) terms as defined using the DAVID resources.

Analysis of mRNA expression using quantitative PCR

To verify the results of the array analysis, and to analyze differences in mRNA expression after treatment with moisturizers, 34 genes were chosen for analysis with qPCR. Reverse transcriptase PCR was performed using cDNA, using TaqMan® Gene Expression Assays (Life Technologies) or SYBR Green detection.

The following genes were analyzed in healthy controls and in patients with AD/IV at baseline: integrin alpha-3 (ITGA3), catenin (cadherin-associated protein) alpha 1 (CTNNA1), Wiskott–Aldrich syndrome protein (WAS), laminin beta 3 (LAMB3), integrin alpha-E (ITGAE), junction adhesion molecule 2 (JAM2), T-cell-specific surface glycoprotein CD28 (CD28), protein-tyrosine kinase 2-beta 4 (PTK2B4), Toll-like receptor 2 (TLR2), and signal transducer and activator of transcription 2 (STAT2).

In the patients with AD/IV, chemokine (C-C motif) ligand 18 (CCL18), Toll-like receptor 4 (TLR4), CD68, phospholipase A2 group IIA (PLA2G2A), epidermal arachidonate lipoxygenase 3 (ALOXE3), peroxisome proliferator-activated receptor gamma (PPARG), and filaggrin (FLG) were analyzed before and after treatment.

STS and FLG were analyzed in all patients with XLRI at baseline.

NLR family pyrin domain containing 10 (NLRP10), laminin C1 (LAMC1), protein phosphatase methylesterase (PPME1), v-myb myeloblastosis viral oncogene homolog (avian)-like 2 (MYBL2), testicular acid phosphatase (ACPT), and MAX gene associated (MGA) were analyzed in the controls at baseline and in the patients with XLRI both at baseline and after treatment.

Fatty acid synthase (FASN), acetyl-CoA carboxylase beta (ACACB), β-glucocerebrosidase (GBA), serine-palmitoyl-CoA transferase 2 (SPTLC2), 3-hydroxy-3-methylglutaryl-CoA reductase (HMGCR), 3-hydroxy-3-methylglutaryl-CoA synthase (HMGCS1), peroxisome proliferator-activated receptor beta (PPARβ), liver X nuclear receptor β (LXRB/NR1H2), interleukin 1 alpha (IL-1α), and involucrin (IVL) were analyzed before and after moisturizing treatment in all patients.

18S ribosomal RNA 1, GAPDH, β-actin, and cyclophilin A were used as reference genes for geNorm normalization.

Moisturizing treatment

The patients took part in a prospective, randomized, investigator-blinded study to compare the effects of moisturizers on skin barrier function. Three
different moisturizers were used (i.e., A, B, and C). All three are registered OTC preparations in Sweden. They were chosen as representatives of the three most common groups of moisturizers, i.e., those containing glycerol, propylene glycol, and urea. Their contents also differ in lipid type and concentration (Table 2).

Table 2. Ingredients in moisturizers A (Miniderm®), B (Propyless®), and C (Canoderm®).

<table>
<thead>
<tr>
<th>Product name</th>
<th>A – Miniderm®</th>
<th>B – Propyless®</th>
<th>C – Canoderm®</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vendor</td>
<td>ACO Hud</td>
<td>MSD</td>
<td>ACO Hud</td>
</tr>
<tr>
<td>Moisturizing component</td>
<td>Glycerol 20%</td>
<td>Propylene glycol 20%</td>
<td>Urea 5%</td>
</tr>
<tr>
<td>Lipid content</td>
<td>23%</td>
<td>9%</td>
<td>22%</td>
</tr>
<tr>
<td>Other ingredients</td>
<td>Hydrogenated canola oil, Cholesterol, Glycerol monostearate, Macrogol stearate, Cetostearyl alcohol, Dimethicone, Light liquid paraffin, Hard paraffin, Petrolatum, Propyl parahydroxybenzoate (E 216), Methyl parahydroxybenzoate (E 218), Purified water</td>
<td>Carbomer, Xanthan gum, Glycerol, Benzyl alcohol, White soft paraffin, Cetyl alcohol, Stearic acid, almitic acid, Glucamate SSE-20, Glycerol monostearate, Sodium hydroxide, Aluminium-magnesium silicate, Dimethicone, Silicone, Purified water</td>
<td>Fractionated coconut oil, Polysorbate 60, Cetostearyl alcohol, Hydrogenated canola oil, Propylene glycol, Carbomer, Dimethicone, Hard paraffin, Glycerol polymethacrylate, Propyl parahydroxybenzoate (E 216), Methyl parahydroxybenzoate (E 218), Sodium lactate solution, Lactic acid, Glyceryl stearate, Polyoxyethylene stearate, Purified water</td>
</tr>
</tbody>
</table>

The patients were randomized with respect to: i) one of three treatment combinations, i.e., A+B, A+C, or B+C; ii) right or left forearm; and iii) order
of inclusion in the study. Each patient applied two different moisturizers, one on each volar forearm, twice daily for four weeks. During the study period, they were not allowed to use any other treatment on their forearms, including skin-care products. Treatment was terminated after four weeks (±three days). On the following day, total and local SCORAD, dryness score, TEWL, and skin pH were measured, and punch biopsies obtained, from both volar forearms (for details, see above). The amount of moisturizer used was determined by weighing each jar before and after the treatment period.

Statistics

Differences in TEWL, pH, and relative mRNA expression between untreated patients and controls were calculated using the Mann–Whitney non-parametric test. Differences in the baseline values of total SCORAD were analysed using Student’s test, and in the baseline values of local SCORAD, dryness score, TEWL, and skin pH using Kruskal–Wallis one-way ANOVA and Dunn’s multiple comparison test.

To identify genes, examined in the microarray analysis, that were differentially expressed between patients and controls, a two-way analysis of variance (ANOVA) between each patient group and the healthy control group was performed using Partek Genomics Suite 6.4 (Partek Inc., St. Louis, MI). For genes chosen for pathway analysis, significance was corrected using Bonferroni multiple testing. To verify altered genes identified by means of quantitative real-time PCR, the relative mRNA expression and statistical significance were calculated using REST 2009 software (available at www.qiagen.com) with Fisher’s exact test.

Differences in the effects of treatments on local SCORAD, dryness score, TEWL, and pH were analyzed using the non-parametric Wilcoxon matched-pair signed-rank test. The mRNA expression after treatment, versus baseline values, was statistically analysed using the non-parametric Wilcoxon signed-rank test in paper III and by one-way ANOVA followed by the Tukey multiple comparison test in paper IV. Moisturizing cream consumption was analysed using Kruskal–Wallis one-way analysis of variance and Dunn’s multiple comparison test.

All tests were performed using Prism 5.04 software (GraphPad Software Inc., La Jolla, CA).
Results

Papers I, III, and IV

Baseline characteristics

Clinical and biophysical measurements

Table 3 shows the baseline values of total SCORAD, local SCORAD, dryness score, TEWL, and skin pH in all patients and healthy controls. The mean SCORAD value for all 43 patients with AD/IV was 12.5, which corresponds to mild AD. Only four patients, two in the FLG+/− group and two in the FLG−/− group, had SCORAD values of 25–50, corresponding to moderate disease activity. Total SCORAD was higher in patients with FLG mutations (FLG+/− and FLG−/−) than in the FLG+/+ group ($p = 0.02$).

The highest dryness scores, values of 2 or 3, were much more common in the FLG−/− group (53.3%) and XLRI group (42.8%) than in the FLG+/+ group (7.1%).

In the case of TEWL, it was confirmed that patients with FLG−/−, FLG+/−, and STS− had higher TEWL than did the healthy controls. Patients carrying single or dual FLG mutations had significantly higher TEWL values than did patients with AD without mutations.

The FLG−/− group had a slightly higher skin pH than did the healthy control group.
Table 3. Baseline values of SCORAD, dryness score, TEWL, and skin pH in healthy controls and the four patient groups.

<table>
<thead>
<tr>
<th>Patients</th>
<th>Healthy controls ($n = 14$)</th>
<th>FLG+/+ ($n = 28$)</th>
<th>FLG+−/− ($n = 28$)</th>
<th>FLG−−/−* ($n = 30$)</th>
<th>XLRI ($n = 28$)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Mean (range)</td>
<td>Mean (range)</td>
<td>Mean (range)</td>
<td>Mean (range)</td>
</tr>
<tr>
<td>Total SCORAD (a.u.)</td>
<td>7.6 (0-14.7)</td>
<td>15.4 (1.7–33.0)</td>
<td>12.0 (4.7–26.9)</td>
<td>13.0 (1.8–36.0)</td>
<td>11.9 (0–32.8)</td>
</tr>
<tr>
<td>Local SCORAD (a.u.)</td>
<td>6.5 (0-22.9)</td>
<td>13.0 (1.8–36.0)</td>
<td>10.9 (0-32.8)</td>
<td>10.9 (0–32.8)</td>
<td>10.9 (0–32.8)</td>
</tr>
<tr>
<td>Dryness Score ≥2b ($n$ (percent))</td>
<td>2 (7.1)</td>
<td>6 (21.4)</td>
<td>16 (53.3)</td>
<td>12 (42.8)</td>
<td></td>
</tr>
<tr>
<td>TEWL (g m⁻² h⁻¹)</td>
<td>8.6 (4.8–13.6)</td>
<td>10.2 (6.5–17.4)</td>
<td>14.9** (8.1–31.8)</td>
<td>13.8** (7.1–37.6)</td>
<td>14.5** (2.8–30.7)</td>
</tr>
<tr>
<td>pH Mean (range)</td>
<td>4.5 (3.3–5.2)</td>
<td>5.0 (3.8–5.2)</td>
<td>5.1 (4.5–6.4)</td>
<td>5.3* (4.7–6.2)</td>
<td>5.1 (3.9–6.1)</td>
</tr>
</tbody>
</table>

* The FLG−−/− group included three patients with no history of atopic eczema; when these three patients are excluded from the statistical analysis, the mean total and local SCORAD values increase to 13.0 and 12.4, respectively.

b Values for the dryness score are shown as the number (and percent) having a score of 2 or 3. $n$ = number of forearms examined

a.u. = arbitrary unit

* and ** = significance relative to healthy controls
mRNA expression

*FLG* was found to be downregulated in the AD/IV patients selected for microarray analysis, and qPCR verified these results in the whole group of AD/IV patients compared with healthy controls (Fig. 3). The reduction in mRNA correlated with *FLG* mutation status, the lowest expression occurring in the *FLG*–/– group followed by the *FLG*+/– and *FLG*+/+ groups.

![Image](image-url)

*Figure 3.* *FLG* mRNA expression determined using microarray analysis and qPCR. Results are presented as columns, showing mean values for healthy controls and the three *FLG*-genotype subgroups.

The microarrays identified a total of 2292 up- and 2076 downregulated transcripts in the three AD/IV groups. Only 27 transcripts were found to be up- and downregulated in the XLRI group (Fig. 4). In the AD/IV patients, some of the transcripts were uniquely altered in the *FLG*+/+, +/–, and –/– groups, respectively, while others overlapped between the groups (Fig. 5). In the XLRI group, only seven of the 27 altered transcripts overlapped the ones being altered in the AD/IV group (data not shown). Quantitative PCR
analysis verified the results in a selected number of genes in all four patient groups (data not shown).

In the AD/IV group, the transcripts belonged to several different pathways, including inflammation, lipid metabolism, epidermal differentiation, and cell signalling. Numbers of both transcripts and pathways correlated with FLG mutation status and FLG mRNA expression. When analyzing the expression patterns in the XLRI group, no specific pathways were found to be affected.

![Figure 4](image_url)

*Figure 4.* Number of transcripts with altered expression, identified using oligonucleotide array analysis, in the four patient groups versus healthy controls.
Figure 5. Unique and overlapping differentially expressed genes in patients with FLG+/-, FLG+/-, and FLG--.

Effect of moisturizing treatment

Clinical and biophysical measurements

Local SCORAD

Local SCORAD was reduced by treatment in the AD/IV patient group as a whole (Fig. 6a), and this was independent of type of moisturizer used (Fig. 6b) and patient FLG mutation status (Fig. 6c).
Figure 6. The effects of moisturizing treatment on local SCORAD on volar forearm skin versus individual baseline values. Panel (a) shows values for all patients irrespective of the moisturizer used. Results are presented as box plots with the median value as a line across the box, the first quartile value at the bottom, and the third quartile at the top. The whiskers are lines that extend from the bottom and top of the box to the lowest and highest observations, respectively. The mean value is shown as (+). Patients with values considered significant outliers at baseline and/or after treatment are excluded. Panels (b) and (c) show before-and-after plots for the three treatment groups, i.e., A, B, and C (b) and the three different FLG-genotype subgroups (c). The red squares represent significant outliers excluded from the statistical calculations. Statistical analysis was performed using the non-parametric Wilcoxon matched-pair signed rank test.

Dryness score

The dryness score was lower after treatment when considering all patients together. The number of patient arms with the highest dryness score (≥2) was reduced from 36 to 8 (Fig. 7a). The most pronounced effects were seen in the FLG−/− and XLRI groups (Fig. 7b), which had the highest dryness scores at baseline (see Table 3), independent of type of moisturizer.
Figure 7. Panel (a) shows the number of patients having dryness scores of 0–1 and 2–3 at baseline and after treatment, respectively. Panel (b) shows the effect of moisturizing treatment on dryness score on volar forearm skin versus individual baseline values. Dryness score was assessed as 0 = none, 1 = mild, 2 = moderate, and 3 = severe. There are several overlapping data points in (b). Note: The different numbers of patients in Figs. 6–8 are due to differences in the number of outliers between assessments and to a few missing data from three patients, one in each FLG-genotype subgroup. For an explanation of the graph, see Fig. 6.

**TEWL**

No difference was seen when comparing TEWL at baseline and after treatment in the four patient groups considered as a single group. TEWL was, however, significantly lower after treatment when considering the patients with AD/IV as one group. When the results were analyzed with regard to moisturizer type and FLG mutation status, no consistent differences were seen. The XLRI group as a whole, on the other hand, had higher TEWL after treatment, independent of the particular treatment (Fig. 8). However, detailed analysis of this patient group revealed that the different moisturizers had different effects, with higher TEWL after treatment occurring only in patients using moisturizer C; no significant changes, in either direction, were seen with treatments A or B.
Figure 8. The effect of moisturizing treatment on TEWL on volar forearm skin versus individual baseline values. The figure shows paired values for all patients, divided into the four patient groups. For an explanation of the graph, see Fig. 6.

Skin pH

Skin pH did not differ after treatment in any of the patient groups (data not shown).

mRNA expression

There were only minor changes in the mRNA expression after treatment, independent of type of moisturizer used. Despite clearly deviant mRNA expressions in FLG+/− and −/− patients at baseline, few alterations in mRNA expression occurred after treatment (data not shown). A reduction \( p = 0.033 \) of CD68 and an increase \( p = 0.027 \) of HMGCR in FLG−/− were seen in patients using moisturizer C, as well as an increase \( p = 0.027 \) of PPARβ in FLG−/− patients using moisturizer B and a five-fold increase of FASN expression in the XLRI group using moisturizer B.

Moisturizer consumption

After four weeks of daily application on each volar forearm, the patients consumed an average of 77 g of moisturizers A and C and 107 g of moisturizer B \( p < 0.01 \); moisturizer consumption did not differ between the four groups of patients (data not shown).
Paper II

One male patient, with longstanding symptoms of dry, flaky skin diagnosed as IV, was investigated in depth before inclusion in the study, because no FLG mutations were detected and no deletions were found in the coding region of STS using MLPA. The STS gene was therefore directly sequenced. This revealed a novel base pair substitution in exon 3 resulting in a premature stop codon (R90X), probably leading to loss of STS enzyme activity and clinical symptoms of XLRI. The patient was included in the XLRI group.
Discussion

Baseline characteristics

Loss-of-function mutations in FLG are a major predisposing factor for AD in European and Asian populations and are one of the primary genetic causes of IV. Most of the AD/IV patients included in the present study had a mild phenotype: only four of 43 patients had SCORAD values corresponding to moderate disease activity. In general, patients with FLG−/− or +/− had a more severe phenotype with higher SCORAD, dryness score, and TEWL values at baseline than those of FLG+/+ patients.

Interestingly, full-length FLG transcripts were demonstrated, using both microarray analysis and qPCR, to be downregulated in all AD/IV groups versus in healthy controls, i.e., also in AD patients without FLG mutations. There was a mutation-dependent downregulation, with the lowest expression in patients with FLG−/−, followed by FLG+/− and FLG+/+. Other studies have demonstrated that pro-inflammatory cytokines, such as IL-4 and IL-12, may modulate the expression of FLG even in patients without FLG mutations. Even though the skin biopsies in our study were taken from non-eczematous skin, subclinical inflammation could be a valid explanation of our findings. One recent study supports this theory, using microarray analysis to find increased FLG expression, relative to baseline, after topical anti-inflammatory treatment of lesional skin in patients with AD.

Several transcripts and signalling pathways were altered, as identified using oligonucleotide array analysis in AD/IV patients (see Fig. 4). Some were uniquely altered in each group, while others were overlapping between the groups (see Fig. 5). Many of the affected genes are localized to chromosomal regions previously linked to AD. This highlights these regions as important loci for candidate genes involved in AD development. The number of altered transcripts and pathways was correlated with FLG mutation status and FLG mRNA expression in the skin, with 3299 up- or downregulated transcripts in the FLG−/− group, followed by 767 in the FLG+/− group and 312 in the FLG+/+ group. The degree of FLG deficiency, leading to a more permeable barrier, apparently influences the number of up- or downregulated genes involved in inflammatory response and barrier repair. Patients with XLRI displayed similar baseline values in dryness score and TEWL values as did AD/IV patients with FLG−/−, indicating the same degree of disruption in skin barrier function. Despite an indisputable barrier
dysfunction, and virtually indistinguishable clinical features at the site of examination, the number of transcripts displaying altered expression in the XLRI patients was surprisingly low, 27, versus 3299 in the FLG−/− group (see Fig. 4). Only seven of these 27 altered genes overlapped those that also were changed in AD/IV patients. The remaining twenty genes were uniquely altered in XLRI patients and included the reduction of two genes located in the same region on the X-chromosome as the STS gene, implying contiguous deletions of several genes.72

Many of the genes altered in AD/IV patients map to inflammatory response, focal adhesion, extracellular matrix receptor interaction, regulation of actin cytoskeleton, and calcium-signalling pathways. Analogously, in healthy skin, a temporarily disrupted skin barrier function, induced by SLS exposure, is followed by acute compensatory activation of various repair mechanisms, including increased expression of involucrin, TGM-1, and profilaggrin.63,73 None of this was seen in the XLRI group, indicating that the skin barrier defect in XLRI does not trigger an inflammatory response or a compensatory repair mechanism in the same way as do barrier defects in AD/IV or SLS exposure.63,73 Further evidence is that patients with XLRI displayed normal FLG expression, without indication of the downregulation of FLG due to inflammation. This lack of inflammatory response could be because the accumulation of cholesterol sulphate in SC seen in XLRI inhibits serine proteases essential for corneocyte shedding.74 The retention hyperkeratosis that follows will function as a “lid” that helps seal the barrier, without need for compensatory repair mechanisms. This also supports the notion that eczema is a rare complication in patients with XLRI.

Effect of moisturizing treatment

Patients with AD, IV, or XLRI often require continuous therapy with moisturizers to improve skin symptoms. In the present work, treatment with three different moisturizers for four weeks resulted in similarly reduced skin dryness in all patients, and lower local SCORAD in the AD/IV patients, independent of type of moisturizer. The TEWL results, on the other hand, were more controversial. In the AD/IV group, TEWL was slightly lower after treatment with all three moisturizers. This corroborates previous results in studies of healthy controls55 and of patients with AD.51 Patients with AD have displayed improved barrier function, measured as decreased TEWL and fewer relapses of eczema, after treatment with a urea-containing moisturizer.51,52 In addition, glycerol-containing moisturizers have been demonstrated to reduce TEWL in patients with AD.75

In contrast, TEWL was higher in the XLRI group after moisturizing treatment, considering both all moisturizers together and the urea-containing moisturizer (C) alone. This is another argument for the skin barrier defect in
XLRI patients differing in nature from that in AD/IV patients. Higher TEWL after treatment with moisturizers suggests that the removal of corneocytes further degrades the barrier function in XLRI. On the other hand, urea-containing moisturizer did not affect TEWL in a study of patients with lamellar ichthyosis, which, however, may be explained by a completely different aetiopathogenesis. Another moisturizer, containing lactic acid and propylene glycol, significantly increased TEWL in lamellar ichthyosis patients, presumably as an effect of more efficient corneocyte removal. In this, and in our present studies, all moisturizers improved skin dryness equally.

Previous studies of healthy volunteers have demonstrated that certain lipid metabolism enzymes, nuclear receptors, and lipoxygenases have both positive and negative effects on mRNA expression after treatment with moisturizers. Therefore, more pronounced effects on mRNA profiling were expected in FLG-deficient skin in terms of dryness, increased TEWL, and many altered epidermal gene expressions at baseline. Surprisingly, the effects of all three moisturizers were quite small in all patient groups. Virtually no changes in the genes selected as biomarkers of epidermal inflammation, lipid metabolism, and barrier repair were detected using qPCR analysis before versus after treatment. The few significant changes in gene expression noted in paper III were detected in the FLG−/− group. This group also displayed the most pronounced differences in SCORAD and dryness score before and after treatment. Although one might speculate that these results relate to patient FLG status, the FLG−/− group was slightly larger than the others, and more outliers were detected in the other two groups, which could have affected the statistical analysis. Furthermore, because of the many analyses (i.e., four patient groups + three treatments + several genes) and the given significance level, false positives are a possibility. Admittedly, since the number of assessed mRNAs was limited, differences between treatments might have been missed, because other genes, not studied here, may be altered by treatment.

Hypothetically, the frequent use of moisturizers containing standard concentrations of glycerol, propylene glycol, or urea might induce a “vicious circle”, whereby the downregulation of lipid-synthesizing genes further damages the skin barrier. However, there was no convincing evidence that moisturizers adversely affected gene expressions involved in keratinocyte differentiation and lipid formation in patients with AD, IV, or XLRI, which stands in contrast to the situation in healthy controls. Although this is reassuring per se, if the altered epidermal gene expression in skin carrying FLG mutations could be normalized by daily applications of any of these three moisturizers, this would of course represent a more ideal situation.

The present study focused on the most commonly prescribed moisturizers in Sweden, containing glycerol, propylene glycol, or urea. Studies of other compounds, such as lipids, could be informative. Two of our studied genes,
GBA and SPTLC2, are essential for ceramide biosynthesis. Interestingly, the lipid composition of SC in AD is characterized by low levels of ceramides.\textsuperscript{77} Specific replacement therapy with ceramide-containing moisturizers has produced positive results in terms of improved SCORAD and reduced TEWL,\textsuperscript{78-80} so this approach merits further attention.

Conclusions

In general, patients with AD, IV, or XLRI display impaired skin barrier function compared with that of healthy controls when assessed in terms of TEWL. The increase in TEWL was similar in patients with IV or XLRI, two skin disorders with difficult-to-distinguish clinical pictures. Despite this, the difference in the number of differently expressed genes was surprisingly large between the AD/IV group and the XLRI group at baseline. These findings emphasize that patients with similar clinical pictures can have different genetic backgrounds. This understanding is strengthened by our finding of a novel mutation in the STS gene in a male patient, previously diagnosed with IV, by sequencing the entire gene. This finding also highlights the importance of further DNA sequencing if the clinical picture is that of IV or XLRI, but no mutations are detected using standard methods.

Moisturizing treatment had a positive effect on local SCORAD in patients with AD/IV and on skin dryness in all patients. Patients with FLG\textsuperscript{−/−} and XLRI – with the highest dry skin scores at baseline – benefitted the most from moisturizing treatment. However, the treatment had no major impact on gene expression profile, and did not seem to normalize the altered epidermal gene expression profile in FLG-deficient skin.

Since none of the moisturizers produced markedly “better” or “worse” effects than the others, it can be concluded from this study that the choice of moisturizer should be individualized, based primarily on factors such as self-perceived improvement, cosmetic considerations, and the cost to patient and society.

Future perspectives

More precise knowledge about the effects of moisturizers on the skin barrier function, it is hoped, could lead to better tailored topical treatment. One interesting aspect would be to study mRNA expression using oligonucleotide array analysis before versus after moisturizing treatment in patients with impaired skin barrier function attributable to different causes. As UV treatment is another common treatment for patients with AD, another interesting perspective would be to examine the effects of UV treatment on mRNA expression in patients with AD. As one of FLG’s decomposition
products, UCA, is important for UV protection, it would be of interest to examine whether response to treatment correlates with \( FLG \) mutation status.
Hudbarriärens funktion och genuttryck hos patienter med atopisk dermatit, ichthyosis vulgaris och könsbunden iktyos – skillnader i sjukdomsorsaker och effekten av mjukgörande behandling

Hudens barriär skyddar oss från uttorkning och från potentiellt skadliga faktorer utifrån. Den återfinns huvudsakligen i det allra yttersta lagret av epidermis (överhuden), hornlagret.

Atopiskt eksem (AD), ichthyosis vulgaris (IV) och könsbunden iktyos (XLRI) är hudsjukdomar som i olika grad kännetecknas av nedsatt hudbarriärfunktion. Den genetiska bakgrunden skiljer sig åt; AD och IV är relaterade till mutationer i den gen som kodar för filaggrin (FLG), ett protein viktigt för hornlagrets uppbyggnad och för dess fuktbindande förmåga. XLRI orsakas av förändringar i STS, den gen som kodar för steroidsulfatas. Brist på detta enzym leder till ansamling av kolsterolsulfat i huden med hyperkeratos (förtjockat hornlager) som följd. Gemensamt för patienter med AD, IV och XLRI är torr hud, och att de ofta använder mjukgörande behandling regelbundet för att lindra sina hudsymptom. Kunskapen om mjukgörande preparats effekter på hudbarriären är dock begränsad.

I de här avhandlingsarbetena har objektiv scoring av hudsymptom, icke invasiva mätmetoder och analyser av hudbiopsier kombinerats för att karaktärisera hudbarriärens funktion och studera effekten av mjukgörande behandling hos patienter med AD, IV och XLRI. 57 patienter delades in i grupper efter analys av FLG och STS mutationsstatus: AD med FLG+/+(n=14), AD med FLG+/− (n=14), AD/IV med FLG−/− (n=15) och XLRI med STS− (n=14). I STS− gruppen inkluderades en patient där vi upptäckte en hittills inte beskriven punktmutation i STS. Även 14 friska kontroller rekryterades till studien.

Patienternas hudsymptom graderades med hjälp av objektiv scoring. Hudbarriärfunktionen undersöktes både med fysikaliska mätmetoder i form av transepidermal vattenförlust (TEWL) och hud-pH, och molekylärbiologiska metoder med analys av genexpression med microarrayanalyser och qPCR. Undersökningarna av patienterna, med undantag för microarrayanalyserna, gjordes före och efter en fyra veckor
lång behandlingsperiod med mjukgörande preparat. Tre olika mjukgörare användes, en med glycerol, en med propylenglykol och en med urea. Patienterna randomiserades till att använda två av dessa, en på varje underarm, två gånger dagligen under behandlingstiden.

Resultaten av studien bekräftar att patienter med AD, IV och XLRI har en nedsatt hudbarriärfunktion mätt som torrhet i huden och ökad TEWL jämfört med friska frivilliga. Hos AD/IV-patienterna sågs att sjukdomens svårighetsgrad och TEWL korrelerade till FLG- mutationsstatus; patienter med FLG+/- och FLG–/– hade svåraare symptom och högre TEWL-värde jämfört med för FLG+/+ gruppen. Även mRNA uttryck av FLG i huden korrelerade till FLG- mutationsstatus, med lägst FLG uttryck i –/– gruppen. Hos patienter med AD/IV sågs även upp- eller nedreglerat genuttryck i ett flertal transkript vid microarrayanalys. Antal transkript med förändrat uttryck korrelerade till FLG–status, med 312 förändrade transkript i FLG+/+ gruppen jämfört med 3299 i FLG–/– gruppen. Flera signalvägar, kopplade till bland annat inflammations- och lipidmetabolism, uppvisade förändringar, framförallt i FLG–/– gruppen.

Trots likartade värden avseende torrhet och TEWL, och en tydlig klinisk bild, sågs förändrat uttryck i förväntansvärt få gener i XLRI gruppen – endast 27 stycken – och inga förändrade signalvägar kunde identifieras.

Samtliga mjukgörare förbättrade torr hud på likartad vis i alla patientgrupper, men effekten på TEWL skiljde sig mellan grupperna. Efter behandling var TEWL lägre i AD/IV gruppen, medan det var högre i XLRI gruppen. Endast minimala effekter sågs på hud-pH och mRNA-expression.

Sammanfattningsvis resulterar mutationer i FLG i ett påslag av gener med mål att reparera skadan i hornlagret. Detta innefattar även ett ökat påslag av inflammatoriska gener, med en ökad risk för eksem som följd. Detta reaktionsmönster ses däremot inte hos patienter med XLRI, förmodligen beroende på att den hyperkeratos de utvecklar ”tätar” hudbarriären utan behov av reparativt genpåslag. Detta ses även kliniskt, eksem är ovanligt hos patienter med XLRI, trots den torra huden.

Mjukgörande behandling hade positiv effekt på torrhet i huden hos samtliga patienter, men, till skillnad från hos patienter med AD/IV, ökade TEWL hos patienter med XLRI efter behandling. Detta kan tala för att hudbarriärfunktionen försämras när hyperkeratosen tunnas ut av behandlingen. Behandling med mjukgörande preparat verkar inte kunna normalisera det förändrade epidermala genuttrycket som finns hos patienter med FLG- relaterad hudsjukdom. Å andra sidan verkar behandlingen inte heller ha negativa effekter på uttryck av gener viktiga för hudbarriärens funktion och för lipidmetabolism i epidermis hos patienter med AD, IV eller XLRI.

Inget i studien tyder på att någondera mjukgörande preparat har markant ”bättre” eller ”sämre” effekter än någon annan. Detta talar för att valet av
mjukgörande behandling måste baseras på andra faktorer såsom kosmetik, patientens egna preferenser och kostnad för patienten/samhället.
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