EPIDERMAL DIFFERENTIATION & THE COMMON ICHTHYOSES

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DEDICATION

For when I grow, I want to be
Higher than high, and better than best
And it is you who will push me up when I am at high
And who will push me forward when I think I am best

To the Source of the universe, who has given me a happy life, who has made my obstacles always small or has given me wonderful friends to help me jump the hurdles

To my parents, Nancy and Antonio, who have rejoiced in my achievements and mourned with me in my defeats, but whom overall have never doubted in my capabilities

To my brother and sister, whom I admire for their achievements as well, and for the maturity they have, which one day I hope to achieve somehow

To Ivan Antonio, who is so little he does not know he was the motor that led me to finish the thesis in due time to haste and meet him, and hold him in my arms

To Leonardo, whose love has been unconditional, who has always fought for ‘us’ when I thought the best was to just be ‘me,’ who does small acts of kindness every day which I don’t even know about, who is a beautiful soul with whom I want to spend the rest of my life.
ACKNOWLEDGEMENTS

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I would like to acknowledge first the government of the Republic of Ecuador, and its National Institution of Higher Education, Science, Technology and Innovation (SENESCYT), who believed I would be a worthy representative of my beautiful country, and whose financial support is what has led me thus far.

I must thank Dr. Rebecca Porter, who still believed in me and in this wonderful project, even when its complexity discouraged me from going on. This has required a lot of her time, effort, and patience. I truly value it all.

I must thank the team at Glamorgan House, especially Dr. Ausama Atwan, Dr. Jui Vyas, Dr. Faraz Ali, Jake and Kaileigh, who have always been eager to help me in times of need and to offer invaluable advice that will be helpful for my lifetime. I also want to acknowledge all my international friends from Glamorgan House. It has been a year I will not forget.

Finally, I also have to mention Osvaldo Guayasamin, one of Ecuador’s most famous painters, whose artwork is at the beginning of every chapter, and which reflect the Indian roots of Latin America that most of my people have tried to forget and oppress throughout times.
DECLARATIONS

This work has not previously been accepted in substance for any degree and is not concurrently submitted in candidature for any degree.

Signed ................................................... (candidate)       Date ............................

STATEMENT 1
This thesis is being submitted in partial fulfilment of the requirements for the degree of MSc in Clinical Dermatology

Signed ................................................... (candidate)       Date ............................

STATEMENT 2
This thesis is the result of my own independent work/investigation, except where otherwise stated. Other sources are acknowledged by explicit references.

Signed ................................................... (candidate)       Date ............................

STATEMENT 3
I confirm that the electronic copy is identical to the bound copy of the dissertation

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I hereby give consent for my thesis, if accepted, to be available for photocopying and for inter-library loan, and for the title and summary to be made available to outside organisations.

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<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>APC</td>
<td>Antigen Presenting Cells</td>
</tr>
<tr>
<td>SC</td>
<td>Stratum Corneum</td>
</tr>
<tr>
<td>LC</td>
<td>Langerhans Cells</td>
</tr>
<tr>
<td>KCs</td>
<td>Keratinocytes</td>
</tr>
<tr>
<td>SS</td>
<td>Stratum Spinosum</td>
</tr>
<tr>
<td>CE</td>
<td>Cornified Envelope</td>
</tr>
<tr>
<td>SB</td>
<td>Stratum Basale</td>
</tr>
<tr>
<td>KIF</td>
<td>Keratin Intermediate Filaments</td>
</tr>
<tr>
<td>ECM</td>
<td>Extracellular Matrix</td>
</tr>
<tr>
<td>TACs</td>
<td>Transiently Amplifying Cells</td>
</tr>
<tr>
<td>AJ</td>
<td>Adherens Junction</td>
</tr>
<tr>
<td>K</td>
<td>Keratin</td>
</tr>
<tr>
<td>TJs</td>
<td>Tight Junctions</td>
</tr>
<tr>
<td>KhG</td>
<td>Keratohyalin Granules</td>
</tr>
<tr>
<td>FLG</td>
<td>Profilaggrin</td>
</tr>
<tr>
<td>SG</td>
<td>Stratum Granulosum</td>
</tr>
<tr>
<td>UCA</td>
<td>Urocanic Acid</td>
</tr>
<tr>
<td>P5C</td>
<td>Pyrrolidone-5-Carboxylic acid</td>
</tr>
<tr>
<td>UV</td>
<td>Ultraviolet</td>
</tr>
<tr>
<td>TGs</td>
<td>Transglutaminases</td>
</tr>
<tr>
<td>SPR</td>
<td>Small Proline-Rich Proteins</td>
</tr>
<tr>
<td>LB</td>
<td>Lamellar Bodies</td>
</tr>
<tr>
<td>STS</td>
<td>Steroid Sulphatase</td>
</tr>
<tr>
<td>CS</td>
<td>Cholesterol Sulphate</td>
</tr>
<tr>
<td>CD</td>
<td>Corneodesmosome</td>
</tr>
<tr>
<td>KLKS</td>
<td>Kallikreins</td>
</tr>
<tr>
<td>LEKT1</td>
<td>Lymphoepithelial Kazal-type related inhibitor</td>
</tr>
<tr>
<td>PKC</td>
<td>Protein Kinase C</td>
</tr>
</tbody>
</table>
- OMIM.............................. Online Mendelian Inheritance in Man
- IV ....................................... Ichthyosis Vulgaris
- XLI ..................................... X-linked Ichthyosis
- lod ...................................... Logarithm of the Odds
- AD ...................................... Atopic Dermatitis
- OR ...................................... Odds Ratio
- TEWL ................................. Trans Epidermal Water Loss
- ADHD .................................. Attention Deficit Hyperactivity Disorder
- DSMV-IV .............................. Diagnostic and Statistical Manual of Mental Disorders, 4th revision
- QoL ..................................... Quality of Life
- CI ........................................ Congenital Ichthyosis
- NHP ..................................... Nottingham Health Profile
- IQoL ..................................... Ichthyoses Quality of Life
- DLQI ..................................... Dermatology Life Quality Index
- SF-12 ..................................... Short Form-12 health-related questionnaire
- RCT ..................................... Randomised Controlled Trial
- kDa ...................................... Kilo Daltons
- DHEAS ................................. Dehydroepiandrosterone Sulphate
- cDNA ..................................... Complementary Deoxyribonucleic Acid
- pSTS ..................................... STS Protein expression vector
- rhSTS ................................. Recombinant Human Steroid Sulphatase
- 1UA ..................................... One Unit of Activity
- ISG ..................................... Ichthyosis Support Group
- FIRST ................................. Foundation for Ichthyosis and Related Skin Types
- CHILD ................................. Congenital Hemidysplasia With Ichthyosiform Erythroderma And Limb Defects
ABSTRACT

The differentiation of the epidermis is a remarkable process that takes place in around 28 days and renders a mesh of corneocytes surrounded by hard shells. This mesh of dead keratinocytes is arrayed in a matrix of lipids that form a barrier which must also allow external communication. Disorders of cornification can arise in any of the processes that transform a basal cuboidal, nucleated cell to the flattened remnant seen in the stratum corneum. One family of these important conditions are the ichthyoses. The ichthyoses are comprised of over 30 skin diseases that vary in pathophysiology, molecular abnormality and phenotype; yet they are grouped under one umbrella of fish-like scaly skin. This review comprehends the process of epidermal differentiation, to then focus on the epidemiology, clinical features, and management of the two most common ichthyoses: ichthyosis vulgaris and X-linked ichthyosis. Traditional treatments for these skin conditions are inadequate and not focused to the particular disease. Therefore, this thesis has proposed a novel pathogenesis-based treatment for X-linked ichthyosis. It is hoped that this review will revamp the interest in these disorders of cornification and that it will underscore the importance of developing new treatments and treatment delivery methods. This work also highlights the significance of improving physician and patient education to better the management and the quality of life of all those patients living with ichthyosis.
Chapter 1

INTRODUCTION
Chapter 1:

1.1 INTRODUCTION

The skin is the largest organ of the human body; it is both a barrier for protection and a lieu for communication with the surroundings through the sense of touch. The integument provides protection against physical, chemical, and microbiological insults. The skin is also a site of homeostasis, as it aids maintaining the balance of temperature, electrolytes, and water in our body. Patterns of skin communicate with the environment, playing a role in interpersonal relationships and courtship; for instance soft, smooth skin, colour-coated nails, long and shiny hair, and facial features enhanced through make up capture the attention of the passer-by to someone’s skin. Moreover, the skin is a window to portray changes in the function of inner organs such as the liver (jaundice), the lung (pink puffers or blue bloaters), or the kidneys (uremic frost). In addition, the integument is a site for hormonal and vitamin synthesis, and it is an important part of the adaptive immune response. These functions portray the significance of this organ, and why the skin is still such a highly researched topic.

The skin is formed by the epidermis, the dermis, and the subcutis (Figure 1). The uppermost epidermis is made up of four different strata; it is self-renewable and takes about 28 days of migration and metamorphosis from a basal cell to a shedding corneocyte. The epidermis does not have blood vessels, but it is nourished by simple diffusion from the dermis; this section is also responsible for the individual skin-colour depending on activity of melanocytes and size of melanosomes. The epidermis also holds Merkel cells, important for the sense of touch; and Langerhans cells, which are antigen presenting cells (APC) that can become stimulated if an external insult surpasses the barrier formed in the stratum corneum (SC). Most Langerhans cells (LCs) are located in the stratum spinosum and play a part in autoimmune diseases, where the body loses capability to recognise self from foreign antigens. Furthermore, the epidermis, especially its structure, is important for therapeutics as it is both available for topical application, but also an impenetrable barrier that needs to be traversed for drug delivery.
To provide an anchor between the basal cells of the epidermis and the upper dermis there is a basement membrane with attachment of several structures from both sides. The loss of this attachment gives rise to blistering diseases such as bullous pemphigoid and epidermolysis bullosa.

The dermis is composed of fibroblasts that produce collagenous connective tissue. The dermis also holds blood vessels and nerves, the annexes of the skin, and members of the immune system like macrophages and dermal dendritic cells. Two regions make up the dermis: an upper papillary and a lower reticular segment. The papillary dermis locates below the basement membrane and projects into tips that shape a wavy outline known as the rete pattern (Figure 1); it also holds terminal capillaries that allow the diffusion of nourishment for the epidermis. The lower, reticular dermis is composed of dense collagen, fibroblasts, blood vessels, nerves, the skin annexes such as the hair follicles and the sweat glands, amid other structures.

The hypodermis or subcutis is debated as to whether it belongs to the skin or to the adipose tissue. However, it is a layer interconnected to the dermis sharing vasculature, hair follicles, and fibroblasts. Yet the main component of the subcutis are adipocytes that aid in thermogenesis and in hormonal synthesis. According to
Driskell and colleagues\(^\text{13}\) adipocytes are not exclusive to subcutaneous tissue and they can also be found within the dermis.

The main cells of the epidermis are the keratinocytes (KCs); and they must traverse a changing journey to fulfil the epidermal roles previously described. Once detached from the basement membrane, the KCs leave the stratum basale, and start an upward migration through the stratum spinosum (SS), granulosum and corneum (Figure 1). The stratum basale is a monolayer of KCs attached to the basement membrane, its cells replicate and some move upwards for differentiation.\(^\text{15}\)

The stratum spinosum owes its name to each cell’s star-like projections, that end at intercellular junctions known as desmosomes.\(^\text{16}\) The KCs in the granular layer portray a cytoplasm dotted with granules that contain elements needed for cornification. Finally in the SC, the keratinocytes have lost the intracytoplasmic organelles,\(^\text{17}\) flattened their structure, and replaced the cell membrane with a cornified envelope (CE) surrounded by lipids.\(^\text{18}\)

Although all regions of the integument are equally important, common dermatoses have great impact on the structure of the epidermis; for example, hyperproliferation and scaling in psoriasis and ichthyosis, blistering and lichenification in different dermatitis, occlusion of hair follicles in acne vulgaris, ulceration and pigmentation produced by skin cancer.

Some skin diseases produce abnormalities in keratinocyte differentiation, and are known as disorders of cornification. These disorders of cornification include the family of ichthyoses, composed of over 35 conditions.\(^\text{19,20}\) The most common ichthyoses are ichthyosis vulgaris and X-linked ichthyosis (Figure 2). Ichthyosis vulgaris causes an abnormal skin barrier so the epidermis proliferates excessively to attempt its repair.\(^\text{21}\) Opposed to this concept, in X-linked ichthyoses the normal scales of the skin do not desquamate properly so they are retained one on top of another giving the clinical appearance of hyperkeratosis (Figure 2).\(^\text{22}\)

The group of ichthyoses are rare and thus limited by a narrow range of therapies which include moisturisers, retinoids, and vitamin D analogues, among other few disease-specific therapies. Therefore research is increasingly focusing on developing treatment options and delivery methods that would benefit these patients, and which can be also utilised for more common skin conditions.
This review focuses on the journey and transformation the KCs undertake from the basal layer to the surface of the epidermis. This work then describes ichthyosis vulgaris and X-linked ichthyosis. The thesis will then propose a novel therapy for the treatment of X-linked ichthyosis, which targets the physiopathology of the condition. It is hoped that this work underscores the significance that the ichthyoses have in the medical field and especially in the dermatological practice.

Figure 2. Image of the scales in ichthyosis vulgaris and X-linked ichthyosis. Adapted from Krug et al.21
Chapter 2

THE DIFFERENTIATION OF THE EPIDERMIS
Chapter 2:

2.1 EPIDERMAL DIFFERENTIATION

Past experiments indicate that epidermal cells take about 28 days from the stratum basale (SB) to the SC, including 13 days being a nucleated keratinocyte\(^6\) and 13 to 14 days as a corneocyte.\(^23\) The cells that undergo differentiation must follow various steps that render a flattened remnant without organelles or a plasma membrane; yet, this remnant is surrounded with a strong CE, securely adhered to its neighbouring corneocytes and embedded into lipids to form an appropriate barrier.\(^18\) The steps of the epidermal differentiation process are summarised in Table 1 and, although listed as steps, some occur concurrently. The table also shows the stratum where the process takes place as well as elements of interest which are located in a particular stratum; some steps may begin in one epidermal layer and finish in a different one. Unfortunately the time frame on which these events occur is only partially known.\(^6,24\)

<table>
<thead>
<tr>
<th>STRATUM</th>
<th>PROCESS OF DIFFERENTIATION TAKING PLACE</th>
</tr>
</thead>
<tbody>
<tr>
<td>BASAL</td>
<td>• Proliferation and detachment from the basement membrane&lt;br&gt;• Synthesis of K5/K14 keratin filaments</td>
</tr>
<tr>
<td>SPINOSUM</td>
<td>• Massive increase in intercellular connections: adherens junctions and desmosomes&lt;br&gt;• Creation of K1/K10 keratin filaments&lt;br&gt;• Initiation of keratohyalin granule and lamellar body production and packaging&lt;br&gt;• Synthesis of cornified envelope elements</td>
</tr>
<tr>
<td>LOWER GRANULOSUM</td>
<td>• Peak in synthesis of keratohyalin granules and lamellar bodies&lt;br&gt;• Start of formation of the cornified envelope</td>
</tr>
<tr>
<td>UPPER GRANULOSUM</td>
<td>• Peak in calcium that drives the release of granules&lt;br&gt;• Exocytosis of lamellar bodies&lt;br&gt;• Aggregation of keratin filaments with filaggrin&lt;br&gt;• Formation of the cornified envelope&lt;br&gt;• Formation of the lipid envelope&lt;br&gt;• Disintegration of all the intracellular organelles including the nucleus&lt;br&gt;• Creation of tight junctions</td>
</tr>
<tr>
<td>LOWER CORNEUM STRATUM</td>
<td>• Lipid lamellae formation&lt;br&gt;• Post processing of lipids&lt;br&gt;• Desulphatation of cholesterol sulphate&lt;br&gt;• Formation of the corneodesmosomes&lt;br&gt;• Cells remain as flattened remnant with a hard cover</td>
</tr>
<tr>
<td>COMPACTUM</td>
<td></td>
</tr>
<tr>
<td>UPPER CORNEUM STRATUM</td>
<td>• Cleavage of corneodesmosomes&lt;br&gt;• Acidification of the skin pH&lt;br&gt;• Desquamation</td>
</tr>
<tr>
<td>DISJUNCTUM</td>
<td></td>
</tr>
</tbody>
</table>

Table 1. Steps and Key events of epidermal differentiation classified by strata
2.1.1 BASAL CELLS: ATTACHMENT AND PROLIFERATION

The basal KCs are attached to the basement membrane and proliferate. The cells are attached to the dermoepidermal junction via hemidesmosomes; these structures link the cell’s Keratin Intermediate Filaments (KIF) in the epidermis to collagen fibrils in the dermis. The attachment of the basal cells also helps with an inherent polarity as the apical and basal regions of the KCs are different in structure and function. While the base of the cells is adhered to the extracellular matrix (ECM) via the hemidesmosomes and secretes collagen and laminin V, the apex of the basal KCs has elements that will help rotate the mitotic spindle to ensure the production of daughter cells ready for differentiation.

A second characteristic of the basal KCs is proliferation. The SB is formed of three different cell types: keratinocyte stem cells, transiently amplifying cells (TACs), and post-mitotic differentiating cells. The perpetual stem cells will produce a pool of TACs by symmetric mitosis, a division that will create a daughter cell next to the mother cell. The TACs have a definite number of divisions and a set life span; the TACs can also divide by symmetric mitosis, perhaps used to extend coverage as in wound repair. In addition, the TACs can undergo asymmetric mitosis thanks to a 90-degree rotation in the mitotic spindle (Figure 3); this creates a daughter cell that loses contact with the basement membrane, stops expressing anchoring integrins, and moves upward for differentiation.
2.1.2 THE CYTOSKELETON

The main molecules that shape the cytoskeleton are actin, tubulins and keratins (Figure 4).\textsuperscript{18,31} Actin forms the smallest components, microfilaments that measure 7nm.\textsuperscript{31} Microfilaments shape a skeletal ring around the KC’s periphery, becoming a site where adherens junctions (AJs) will bind\textsuperscript{15} while forming intercellular connections. On the intracellular side, actin will link with micro-motor molecules such as myosin,\textsuperscript{31} creating tension that will make the KC move during differentiation.\textsuperscript{15,32}

Tubulin forms the largest cytostucture: hollow microtubules of about 20nm\textsuperscript{31} that interact with the other skeletal elements.\textsuperscript{15} Microtubules may contribute in spindle reorientation in the basal layer;\textsuperscript{15} they also interact with important elements of intercellular junctions such as desmoplakin.\textsuperscript{33}

Keratins (Ks) form a structure with an intermediate size between microfilaments and microtubules, intermediate filaments, that measure about 10nm (Figure 4).\textsuperscript{31} The intermediate filaments provide a scaffold to shape the cytoskeleton; they promote the KCs’ polarity,\textsuperscript{26,31} and play a role in intercellular and basement membrane connections via desmosomes and hemidesmosomes respectively.\textsuperscript{15}
2.1.3 KERATINS

Keratins, which make up the intermediate filaments (Figure 4), are classified as type I or acidic and type II or basic. In the epidermis, the keratins form heterodimers, meaning that a basic keratin is always paired with an acidic keratin (K), such as K1 and K10, or K5 and K14. In the basal layer the KCs express K5/K14; these keratins shape a cuboidal cell, which is relatively large compared to its differentiating counterparts (Figure 3). Keratins 5 and 14 are also components of the anchoring hemidesmosomes; their importance in anchorage is highlighted by epidermolysis bullosa, a condition where a mutation in the keratin pair results in detachment of the epidermis.

Once the KC moves to the spinous layer, it starts expressing K1/K10 which uses the former keratins as precursors to create new intermediate filaments. The differentiating epidermal cells also express other keratins in smaller proportions, such as K9 in palms and soles, and K2 ubiquitously in the stratum granulosum.

Compared to K5/K14, the new set of K1/K10 have an ability to bind the nucleus to the desmosomes, as well as a capacity to aggregate into polymers, which will be important for the flattening of the cell later on. The intermediate filaments also participate in the cellular migration by desmosomal rearrangement. Other roles of the keratins in the epidermis, underscored in laboratory experiments, are the control of cellular size and change in keratin expression as response to stressors. Keratins are also thought to mediate pathways such as proliferation, differentiation and apoptosis, but this topic is still undergoing research.

During differentiation, the KIF become highly phosphorylated and acquire strong disulphide bonds. The KIF associate with filaggrin at the granular layer forming bundles which then become crosslinked to the cornified envelope.

2.1.4 THE INTERCELLULAR JUNCTIONS OF THE EPIDERMIS

There are four types of intercellular connections in the epidermis: adherens junctions, desmosomes, gap junctions, and tight junctions. The adherens junctions are massively formed in the SS, where they bring cells very close together and sometimes link them, leaving gaps that will be filled with desmosomes.
The desmosomes are the other intercellular link. Desmosomes are a complex structure that have intracytoplasmic and intercellular domains (Figure 5); basically they gather KIF with desmoplakin and other elements to form an intracytoplasmic plaque that is adhered to the KC's membrane, and then, via elements on the intercellular side, join an identical structure in an opposing KC.42,43

![Desmosomal structure representation](image)

Figure 5. a, Desmosomal structure representation; b, Image superposed to an electron microscopy of two neighbouring keratinocytes. Adapted from Green et al.43

Studies point to a role of desmosomes in nuclear disappearance and in KC migration. Lee and colleagues,44 show that the KIF organise in a cage around the nucleus before extending to join the desmosomes in the plasma membrane; this perinuclear cage, along with the connections reorganises before and during nuclear disintegration. Another study that further settles this notion, is that of Wallace et al.45 where the group noticed that K1/K10-null mice showed fewer desmosomes, early nuclear disappearance and an abnormally structured nucleus. This suggests that the desmosomal rearrangement will help with the nuclear loss during differentiation.38 The importance of these junctions in cellular movement is highlighted in wounds, where desmosomes decrease temporarily their adhesiveness to allow KC migration and differentiation for healing.43,46

The third intercellular link are tight junctions (TJs).40 Tight junctions first appear in the granular layer, and are bound to the KCs' actin microfilaments in a similar fashion to AJs.15 Tight junctions act as an extremely selective fence that only allows permeation of
small molecules and ions, and locates on the KC’s apical side in the granular layer. They also participate in immunity by providing a binding site for the dendrites of Langerhans cells.\textsuperscript{15}

Lastly, another important link are gap junctions. These are formed by connexons that create a channel between neighbouring cytoplasms to allow the passage of ions and other small molecules.\textsuperscript{15}

**2.1.5 KERATOHYALIN GRANULES**

Quickly after the expression of K1/K10, and thanks to an increase of intracellular calcium,\textsuperscript{47,48} the KCs begin synthesis of keratohyalin granules (KhG).\textsuperscript{31,49} The two most recognised constituents of the granules are profilaggrin\textsuperscript{31} and loricrin.\textsuperscript{50} Yet, recent studies reveal that there are other constituents such as cystatin-A,\textsuperscript{18} repentin,\textsuperscript{51} and caspase-14.\textsuperscript{52} In order for loricrin and profilaggrin to be packed in the granules, they must be highly phosphorylated.\textsuperscript{18}

The KCs increase the synthesis of these granules progressively until they reach a peak in the granular layer, where the KhG contents are released, dephosphorylated and cleaved into the active molecules.\textsuperscript{18,53} these contents are important for intracellular rearrangement and for the formation of the cornified envelope.\textsuperscript{18}

**2.1.6 THE FILAMENT AGGREGATING PROTEIN**

Filaggrin, starts as profilaggrin, encoded by the gene FLG on chromosome 1q21, a region named the epidermal differentiation complex because it holds many genes to express components of the cornified envelope.\textsuperscript{54,55} When the precursor profilaggrin is synthesised, it is immediately phosphorylated\textsuperscript{56,57} and packed into KhG.\textsuperscript{31} In the transition of stratum granulosum (SG) to SC the granules release their contents, and profilaggrin is dephosphorylated and cleaved by caspase-14.\textsuperscript{56,58} The process produces filaggrin monomers that start packaging the KIF into bundles to compact the KCs.\textsuperscript{56,59}

The new rearrangement of the KIF with filaggrin, renders them parallel to the skin surface so that shearing forces are transmitted horizontally.\textsuperscript{18} The newly rearranged KIF/filaggrin compound is then crosslinked to the CE.\textsuperscript{59} But this is not the only function of this molecule (Figure 6); among other tasks, it appears that filaggrin plays a role in the correct assembly of lamellar bodies and consequently in the correct morphology of the lipid lamellae.\textsuperscript{60} This will be discussed in section 3.2, profilaggrin and ichthyosis vulgaris.
Some of the filaggrin monomers are further processed by caspase-14 to produce amino acids such as serine, glycine, arginine, histidine, glutamine, and alanine, as well as amino acid derivatives like urocanic acid (UCA) and pyrrolidone-5-carboxylic acid (P5C). Some of these amino acids and P5C can hold water, thus contributing to the natural moisturising factor of the skin. Moreover, the low pH of the derivatives contributes to the acid mantle of the SC (Figure 6). This low pH is appropriate for antimicrobial activity and for adequate cellular cohesion in the SC.

Filaggrin is a molecule very rich in histidine; this histidine is processed, as seen in figure 6, to UCA. Urocanic acid may also contribute to the low pH of the utmost epidermal layer, though this is under discussion role since histidine-deficient animals and humans have a normal SC pH. Yet the most important role of UCA is that of an ultraviolet (UV) scavenger that changes configuration when hit by UVB light and causes a decrease in immune response (Figure 6).

![Figure 6. Extracellular roles of filaggrin. Taken from Elias.](image)

The other components of FLG are the N and C terminals (Figure 7). The amino or N-terminal has two portions: a calcium-binding region and a nuclear localisation region. The calcium-binding domain fulfils a regulatory role; indeed calcium seems to regulate filaggrin expression and function, the increase in calcium starts the expression of differentiation markers including filaggrin and other CE components. Moreover, a peak in calcium in the SG, causes the release of profilaggrin from the keratohyalin granules; this underscores the regulatory role of the calcium-binding region. The
portion of FLG that shifts to the nucleus may contribute to its condensation during differentiation.\textsuperscript{57,63,67} As far as the C-terminal of FLG, its function is still unknown.\textsuperscript{59}

Figure 7. Profilaggrin gene. Taken from Brown et al.\textsuperscript{59}

2.1.7 MAINTAINING IMMUNOLOGICAL SURVEILLANCE

The epidermis is not a perfect barrier, it can absorb substances and, if the barrier becomes defective, sometimes it will allow the penetration of pathogens. Consequently, the epidermis maintains an active immune system. Kubo and colleagues\textsuperscript{68} determined that LCs carry out the immune surveillance and develop a response in case of antigens breaching the SC barrier; in their experiment they noticed that the LCs, which mostly reside in the SS are able to send their dendrites up into the granular layer, and anchor these dendrites to the TJs forming a fence in the SG.\textsuperscript{15,68} Immune activity will not take place unless a foreign molecule reaches the top layer of the SG. Yet, another method of activation is by KCs themselves, which can release inflammatory cytokines or pattern recognition receptors, such as interleukin 1, tumour necrosis factor, and toll-like receptors;\textsuperscript{69} all these signals can also activate dendritic migration. Besides LCs, it appears that the skin also handles a small subset of T lymphocytes in a quiescent state\textsuperscript{70} that play a role in wound healing.\textsuperscript{71}
2.1.8 REPLACING THE MEMBRANE WITH THE CORNIFIED ENVELOPE

The cornified envelope is the toughest of all the structures in the SC and cannot be destroyed by denaturant or reducing solutions normally applied to break down the epidermis. Therefore it must be specially stained to appreciate its structure (Figure 8). The synthesis of all CE components begins in the SS. Candet al. have divided the formation of the envelope into three steps: Initiation, Reinforcement and Lipid Cover (Figure 9).

Figure 8. Cornified envelope after extraction with solvents. Taken from Kalinin et al.

2.1.8.1 INITIATION

The initiation stage (Figure 9) takes place in the stratum spinosum, where transglutaminases attach members of the plakin family (envoplakin and periplakin) along with involucrin to the inner side of the plasma membrane near desmosomes. There are nine types of transglutaminases in the human body, of these TG1, TG2, TG3, and TG5 have a role in the epidermis, but the most important for cornified envelope formation is TG1. TGs link the proteins of the CE in strong, insoluble disulphide bonds; the TGs also crosslink the ceramides of the lipid envelope via ester bonds on the outer envelope.

2.1.8.2 REINFORCEMENT

The second stage of the CE formation strengthens the structure of the CE and increases its volume by crosslinking many substrates. Loricrin, which makes up about
80% of the CE,\textsuperscript{77} is released from the KhG and linked to the CE directly or using small proline-rich proteins (SPR) as a bridge (Figure 9). The ratio of SPR: Loricrin varies from 1:100 in trunk epidermis to 1:10 in lips, palms and soles.\textsuperscript{77,78} This has led scientists to believe that the ratio provides different mechanical resilience;\textsuperscript{18,77} for instance, the lips, palms, and soles, with a very high SPR content, are areas which endure high mechanical stress. Henry \textit{et al.}\textsuperscript{54} observed an increase in SPR after UV irradiation of a body site concomitant with epidermal thickening. Yet, the thin lips and the thick soles which have the same ratio of SPR to loricrin implies that other constituents modify the properties of the epidermis in different body areas. This is reinforced by the fact that the loricrin and SPR content is always about 85% of the CE regardless of their ratio.\textsuperscript{18}

The KhG also release filaggrin that bundles the KIF and, the KIF/filaggrin complex is also cross-linked to the envelope.\textsuperscript{72} This way, on the reinforcement stage, the intracellular structure becomes supported and fixed by the sturdy CE. Many other molecules are added to the envelope at this stage, most of which are synthesised in the Epidermal Differentiation Complex,\textsuperscript{54,55} which holds genes such as: FLG, loricrin, involucrin, SPR, repetin, late-cornified envelope proteins, and others.

Figure 9. Cornified envelope formation. Taken from Candi \textit{et al.}\textsuperscript{18}
2.1.8.3 LIPID ENVELOPE

The last stage (Figure 9), which occurs concurrently with the reinforcement stage, consists in framing the CE with a single layer of ceramides that had been contained in lamellar bodies (LB). The ceramides form the limiting membrane of these bodies, so that when the LB fuses with the plasma membrane, these ceramides are easily incorporated to the envelope by TG1 which crosslinks them to involucrin, periplakin, envoplakin, among others. This lipid envelope could serve as a scaffold for the arrangement of the lipid layers, as well as to promote cohesion of the KCs, and to impede penetration of molecules inside the cells.

2.1.9 LAMELLAR BODIES

In the SC, the KCs are embedded in multiple layers of lipids. These lipids are synthesised progressively starting in the upper SS until the granular layer, and packed in lamellar bodies. The LB do not only contain epidermal lipids, but also a wide variety of molecules. These include: enzymes for lipid processing, lipases, proteases for desquamation, inhibitors of the desquamating proteases, corneodesmosin for corneodesmosome formation, antimicrobial peptides, and steroid sulphatase (STS) to form cholesterol.

β-glucocerebrosidase will process glucosylceramides and sphingomyelinase will process sphingomyelin to form ceramides; all of these are extruded from the LB. Additionally, the LB contains phospholipids and phospholipase A2; the phospholipase will convert the phospholipids into free fatty acids and glycerol.

Cholesterol is one of the few stratum corneum lipids which is not packed in the lamellar bodies. Cholesterol in the SG/SC is in the form of cholesterol sulphate (CS), which is a very miscible molecule, and can passively diffuse into the intercellular space. However, steroid sulphatase is contained in the LB, and once extruded, will convert CS to cholesterol.

The rise of extracellular and intracellular calcium in the SG is what drives the exocytosis of the LB at the granular/corneal interface. It is not only the contents of the LB that are of relevance for epidermal differentiation: the limiting membrane of the LB is composed of acylglucosylceramides that, as soon as the LB fuses with the plasma membrane, are incorporated onto the outer side of the CE. This process forms the lipid
envelope. The rest of the contents are secreted into the extracellular space and they arrange into layers or lamellae that form the "mortar" of the skin barrier.

### 2.1.10 INTERCELLULAR LIPIDS

The lipids extruded from LB form layers between the corneocytes. The proportion of lipids in the SC seems to be about 50% ceramides, 25% cholesterol, 15% free fatty acids, and 10% other substances. The smallest percentage include phospholipids, antimicrobial proteins and peptides, lipid-processing and other hydrolytic enzymes, desquamating proteases, and others. These lipids can retain moisture in the SC; in case of barrier disruption, either by solvent application or by tape stripping, it is noticed that more lamellar bodies escape from the cell for barrier restitution. Moreover, the synthesis of cholesterol, triglycerides and phospholipids is immediately increased. Sphingolipids, the precursors of ceramides, are also synthesised, but they are the last ones to recover after barrier injury.

The lipids accomplish other functions aside from barrier. In fact ceramidases act on ceramides to release sphingosine, dihydrosphingosine and 6-hydroxysphingosine, which are potent antimicrobials.

Other lipids are found on the epidermis. The sebum derived from the sebaceous glands is rich in vitamin E with antioxidant properties, triglyceride derivatives such as lauric acid and sapienic acid, which are strong antimicrobials. Glycerol can also be derived from the sebaceous glands, and it has good moisturising properties.

### 2.1.11 THE ACID MANTLE OF THE SKIN

Throughout the epidermis there is a pH gradient which is maintained neutral at a value of 7 until the KCs reach the SG. Above from this section, small lakes of acidity are dispersed between the keratinocytes, until they fill the whole stratum corneum to a pH of about 5.

There are three main components that provide the necessary acidity of the SC. The first one are the free fatty acids which come about from the post processing of phospholipids. The second component is a sodium-proton exchanger which adds hydrogen ions into the intercellular space. According to Hachem and colleagues, the third group of contributors to the low pH are urocanic acid and other amino acids though in a smaller proportion.
The acid pH of the utmost SC is important for antimicrobial and anti-inflammatory activity, as well as for corneocyte adhesion. The acid mantle inhibits the proliferation of pathogenic bacteria such as staphylococcus aureus and streptococcus pyogenes in the skin; it has also been noticed that this acidity of the epidermal surface inhibits inflammatory action. A well-regulated pH is also important for adequate corneocyte cohesion. It is noticed that both extremes of pH can cause desquamation. An acidic pH itself can cause desquamation by dissolving the attachment of the corneocytes; this is noticed in the widespread use of acids as keratolytics in treatment of skin conditions. A basic pH also accounts for increased desquamation; indeed it has been noticed that applying superbases to the surface of the skin results in restoration of protease activity; moreover, historically it has been noticed that baths with the basic bicarbonate in patients with ichthyosis can help decrease the scaling of their bodies.

2.1.12 THE CORNEODESMOSOMES

For a number of years it has been taught that the epidermis resembles bricks and mortar, where the bricks are the corneocytes and the lipids are the mortar that should hold everything together. Yet, modern research shows that this concept does not reflect the structure in the SC. There are corneodesmosomes (CD) dotted on the periphery of the KCs in the upper SG and SC, and it is the CD that really seem to keep the cells together. Indeed Chapman et al. carried out an experiment in which they removed all the stratum corneum lipids in a pig’s ear; if the lipids were holding the KCs together, it was expected that the corneocytes would detach. However, the result was that the KCs, being devoid of the suspension formed by the lipids, increased their cohesiveness.

The corneodesmosomes are modified desmosomal structures that contain desmoglein 1, desmocollin 1 and corneodesmosin. Desmoglein and desmocollin are already in the intercellular space forming part of desmosomes. Corneodesmosin is synthesised in the spinous layer increasingly until the SG where it is packed in the LB, and finally extruded in the SG/SC interface. Once extruded, corneodesmosin attaches to the desmoglea of desmosomes while the desmosomal plaque is crosslinked to the CE. Corneodesmosin is a glycoprotein rich in serine and glycine, with an amino and carboxy-terminal that can form very adhesive Velcro-like glycine loops. The serine content
provides a site for serine-protease degradation. The protein also has a non-adhesive core that sometimes remains with corneocytes after they are shed.

There are major differences between desmosomes and corneodesmosomes, the first difference is that, though desmosomes have an intracellular domain, they are not bound to the plasma membrane; whereas corneodesmosomes are heavily crosslinked to the CE. The second difference is the corneodesmosin which changes the microstructure of the adhesion complex turning it into a single, homogeneous band. The third difference is that, while desmosomal adhesiveness is calcium-dependant, corneodesmosomes adhesiveness is not modified by the presence or absence of this ion.

In the SG the desmosomes are changed into corneodesmosomes, and, according to experiments from Igawa et al. tight junctions bind to either side of corneodesmosomes, preventing the action of the degrading proteases; but the TJs selectively bind to peripheral corneodesmosomes. Therefore, in the upper SC corneodesmosomes are noticed only in the cell periphery, while those corneodesmosomes once located in the centre of the keratinocyte have been degraded. Igawa and colleagues showed that TJs accompany corneodesmosomes until the 7th or 8th layer out of the 10 to 25 layers that the SC possesses. This important finding aids in comprehending how the cells are attached and detached in the upper SC.

### 2.1.13 DESQUAMATION

Desquamation is the final step of keratinocyte differentiation; it should consist in the unnoticed shedding of small groups of corneocytes once others are ready to take their place. Desquamation in the epidermis is naturally a regulated process that alone has promoters and inhibitors. Firstly, the stratum corneum can be divided into the stratum compactum, where corneocytes are still attached together and unable to desquamate, and the stratum disjunctum, which are layers of KCs loosely attached and ready for shedding. The difference in attachment in the two subsets of the SC are provided by the density of corneodesmosomes which, on the upward journey of keratinocytes, are degraded by proteases.

Proteases seem to be important on getting the cell ready for desquamation in the stratum compactum by cleaving corneodesmosomes. There are different families of
proteases in the epidermis such as kallikreins and cathepsins. Kallikreins are serine proteases, meaning that serine is the amino acid they cleave; in a similar fashion, cathepsins are cysteine proteases. Lastly, there are also aspartic proteases in a smaller quantity. All the enzymes are excreted as precursors, and some are contained in the lamellar bodies.

Kallikreins (KLKs) are the most important and earliest recognised family including 15 different serine proteases, of which eight have been located in the epidermis. To this family belong KLK-5 also known as stratum corneum trypsin enzyme, and KLK-7 or stratum corneum chymotryptic enzyme. Kallikrein 5 seems to have a pivotal role in corneodesmosome degradation because this is the only enzyme that can activate itself as well as other KLKs. Matriptase is another activator of serine protease activity.

Protease function is highly regulated by molecular inhibitors and pH. Regarding pH, serine proteases exhibit an optimal neutral pH activity, whereas cystein proteases function best at an acidic pH. This implies that KLKs have a better performance on the lower SC, but experiments of Caubet et al. indicate that KLKs can still cleave CDs in an acid environment. Yet, on the stratum disjunctum, where the pH has decreased, the primordial protease activity seems to be that of cathepsins.

The molecular inhibitors of protease activity include lymphoepithelial Kazal-type related inhibitor (LEKTI) and other leuko-protease inhibitors such as elastin. Of these, LEKTI is crucial as it has 15 different domains able to inhibit KLKs. LEKTI also works best at a neutral pH. Thus, as soon as LEKTI and the proteases are extruded from the LB one starts inhibiting the others. LEKTI preferentially binds to KLK5 to stop altogether serine protease activation. As the pH slowly decreases, it does so by forming small lakes of acidity in the centre of corneocytes where LEKTI stops inhibition and proteases start cleaving corneodesmosomes. Peripheral CDs, seem to be protected of early degradation by tight junctions. Other inhibitors of protease activity include calcium and cholesterol sulphate; an increase in calcium and in cholesterol sulphate will reduce protease activity.

Once the central corneodesmosomes have been degraded, the KCs have reached the stratum disjunctum. Here, cohesiveness is influenced by hydration and by changes in lipid lamellae. A novel experiment by Lin et al. shows that when the epidermis is hydrated, lacunae of fluids create gaps between the lipids of the bilayers; then, when the
skin is dehydrated, the gaps remained filled by air. Moreover, in the stratum disjunctum, ceramidases cleave the ceramides rendering a disorganised lamella of lipids that can detach easier.¹⁰¹

2.1.14 STEROID SULPHATE, CHOLESTEROL, STEROID SULPHATASE

Cholesterol makes up about 25% of lipids in the SC and goes through a cycle of sulphatation and desulphatation in the epidermis (Figure 10).⁸⁴,¹⁰⁵ In the lower epidermal layers, a sulphotransferase, SULT2B1b, adds a sulphate group to cholesterol, so that cholesterol sulphate accumulates in a gradient that reaches a peak in the granular layer.⁸⁴ Thanks to the added sulphate group, CS can easily diffuse in both water and lipids,¹⁰⁶ thus it spreads into the intra and extracellular spaces.¹⁰⁷ In the SG the concentration of CS is normally about 5% of all the lipids.¹⁰⁶ In the SG to SC, steroid sulphatase is extruded from the lamellar bodies; some STS is also located in microsomes within the cytoplasm. This enzyme acts on the CS by taking the sulphate group away, so that free cholesterol can intermix with the other lipids in the extracellular lamellae. Thus, normally, cholesterol sulphate comprises only 1% of the lipids on the SC.¹⁰⁶

Figure 10. The cholesterol sulphate cycle. Taken from Elias et al.⁸⁴

Cholesterol sulphate seems to be ubiquitously distributed in the body; it is found in body fluids, organs and tissues, and it has various roles in tissues which include regulation of body functions, stabilisation of cell membranes, and participation as a substrate for hormonal synthesis, among others.¹⁰⁸
In the epidermis, the cycle of CS has a role in epidermal differentiation. Intracellularly, CS activates several protein kinase C (PKC) enzymes, induces transcription of TG1, and regulates the transcription of involucrin by binding to a promoter region. Denning’s group also shows CS as an inductor of filaggrin and loricrin expression; yet, CS cannot induce complete KC differentiation on its own. Inside the cell, 90% of the CS localises to the plasma membrane where it could also serve to stabilise the KC structure and protect it from lysis, performing the same role CS plays with erythrocytes. The roles of cholesterol sulphate in the epidermis are summarised in figure 1, on the left, in blue.

Once located in the extracellular space, CS provides feedback for cholesterol synthesis. As it can be seen in figure 10, in the lower SC, there are high levels of CS, these high levels provide a negative feedback for cholesterol synthesis and aid in KC cohesion. However, as STS progressively desulphates CS in the SG and SC, the reduced levels of CS promote new cholesterol synthesis in the lower epidermal layers.

The reduced CS in the outer SC also aids in a change towards a more basic pH, as cholesterol is more basic than when sulphated. This provides the desquamating proteases with an optimum pH to shed the KCs. Figure 11 in purple shows a summary of the actions of the desulphated cholesterol with regards to epidermal homeostasis.
Chapter 3

THE COMMON ICHTHYOSES
Chapter 3:

3.1 DISORDERS OF DIFFERENTIATION AND THE COMMON ICHTHYOSES

Damage to epidermal components can give rise to a broad range of diseases. For instance, and starting from proximal to distal, the incorrect connection of the epidermis to the dermis through the hemidesmosomes can produce bullous pemphigoid or epidermolysis bullosa, diseases of detachment that clinically translate into epidermal fragility and blisters.\(^\text{15}\)

Moving upwards, in the stratum spinosum, an antibody-mediated destruction of the desmosomes connecting the cells causes pemphigus vulgaris. Moreover, mutations in the newly expressed keratins, K1 and K10 cause bullous congenital ichthyosiform erythroderma, now classified as epidermolytic ichthyosis (OMIM #113800).\(^\text{20}\) In the SS the synthesis of components of the CE also begins, such as loricrin and transglutaminase; mutations in their genes can produce loricrin keratoderma (OMIM #604117), and lamellar ichthyosis (OMIM #242300) respectively.\(^\text{53,115}\)

Continuing into the stratum granulosum, the partial or total absence of filaggrin is a major risk factor for atopic dermatitis and the cause of ichthyosis vulgaris.\(^\text{59,116}\) In the SG, as the extrusion of the LB starts, a mutation in the transporter involved in the correct assembly and exocytosis of the LB leads to Harlequin ichthyosis (OMIM #242500).\(^\text{22,115}\)

In the intercellular space, within SG and SC, an altered lipid content due to excess of cholesterol sulphate leads to X-linked ichthyosis.\(^\text{117}\) Also, in the intercellular space, the lack of β-glucocerebrosidase inhibits the adequate processing of ceramides in Gaucher disease.

These are only some of many genetic diseases caused by alterations in the components of the epidermis. References from Lopez-Pajares et al.\(^\text{118}\) and from Nishifuji et al.\(^\text{53}\) provide tables with a summary of the many conditions caused by genetic mutations in epidermal structure.

The family of ichthyoses include over 35 conditions affecting the structure and differentiation of the epidermis.\(^\text{20}\) The word ichthyosis derives from the ancient Greek root ἰχθύς (ichthys), which means fish, referring to the fish-like scales on the skin of patients. Though grouped under one umbrella of scales and abnormal keratinisation, the ichthyoses are extremely diverse in cause, pathophysiology and clinical presentation.\(^\text{119}\)
As noted by Shwayder,\textsuperscript{119} there are differences in the thickness, colour, and shape of the scales: softer and lighter in ichthyosis vulgaris, small and dark in X-linked ichthyosis, and plate-like, thicker, and darker in lamellar ichthyosis (Figures 2 and 12). There are also variations of colour and characteristics in the underlying skin including blistering or erythroderma.\textsuperscript{119}

Figure 12. Characteristics of scale in ichthyosis vulgaris, lamellar ichthyosis, and X-linked ichthyosis. Adapted from Shwayder\textsuperscript{119} and Oji and Traupe.\textsuperscript{22}

The range of these diseases is so diverse that physicians met in Sorèze in 2009, to reach a consensus on the classification of the inherited ichthyoses (Appendix 1).\textsuperscript{20} The classification has a subset with the two most common ichthyoses: ichthyosis vulgaris (IV) and recessive X-linked ichthyosis (XLI). Following this, the ichthyoses were divided into whether or not they form part of a syndrome.

The non-syndromic ichthyoses were subdivided into major and minor types. Major types are severe ichthyoses, which impair the whole body. Minor types include ichthyoses partially involving the body, or those which show a severe phenotype at birth but resolve spontaneously such as self-healing collodion baby. Moreover, a separate subset was created for keratinopathies classified as ichthyoses.\textsuperscript{20}
When included in a syndrome, the ichthyoses were classified according to the most severely affected organ other than the skin, thus the group created a subset of ichthyoses with prominent hair abnormalities, another subset of ichthyoses with prominent neurologic signs, and ichthyoses with a lethal course (Appendix 1). Even after all the groups were created, some diseases did not fit into any category and were classified as "other forms" including conditions whose mutation is still unknown.

Though not all ichthyoses are reviewed in this essay, Appendix 1 shows the current classification produced at Sorèze along with the form of inheritance and the genetic mutation for each disease. In some instances one condition can be due to mutations in different genes, as is the case with lamellar ichthyosis or congenital ichthyosiform erythroderma (Appendix 1). This provides an insight on how broad the range of the ichthyotic disorders is.

The two most common ichthyoses, IV and XLI, are caused by different components of the epidermis. IV occurs due to a mutation in the gene encoding profilaggrin. The second most common ichthyosis, XLI, is caused by a deletion of steroid sulphatase. The epidemiology, molecular pathogenesis, clinical features, diagnosis and management of these two important disorders of cornification will be reviewed.
3.2 PROFILAGGRIN AND ICHTHYOSIS

As previously described, profilaggrin serves many roles in the epidermis. It aids in flattening the cell during differentiation, produces natural moisturising factor, produces the UVB scavenger UCA, and aids in maintaining the acidic skin pH (Figure 6).\textsuperscript{62} Mutations in FLG are the inherited cause of ichthyosis vulgaris (OMIM #146700), the most common of the ichthyosis with an incidence of 1 in 250 to 1 in 1,000 individuals.\textsuperscript{120}

Mutation search in FLG represented a challenge for scientists because the gene is quite long and has many repetitive areas.\textsuperscript{56,59} Indeed, 10-12 filaggrin monomers can be encoded by exon 3 of FLG in some individuals (Figure 7).\textsuperscript{63} Though the gene was partially sequenced in 1992,\textsuperscript{121} it was only in 2006 that mutations underlying ichthyosis vulgaris were discovered.\textsuperscript{122}

In 2006, Smith and colleagues\textsuperscript{122} gathered 15 Irish, Scottish and European-American families affected by ichthyosis. Although it was a small cohort, genetic testing proved that they had mutations in FLGR501X or 2282del4. The patients with only one (heterozygous) mutation had a milder phenotype. Patients who had the same mutation in both chromosomes (homozygous), or those who had two different mutations (compound heterozygous) showed a more severe disease.\textsuperscript{122} This study was statistically analysed using logarithm of the odds (lod) score method, which is a tool that can evaluate the possibility of inheriting together two genes which are close to each other, and which may be responsible for a disease; in this method, a value of $Z_{\text{max}}$ above 3 means that the genes are very close to each other and are likely to be passed on together. Smith \textit{et al.}\textsuperscript{122} showed that the genes close to FLG showed a $Z_{\text{max}}$ value of 3.6 with dominant inheritance and $Z_{\text{max}}= 3.4$ in recessive inheritance.

Since the pioneering filaggrin-mutation study\textsuperscript{122} represented a small subset of the population, other studies followed in both similar and different ethnic groups.\textsuperscript{123-125} The research proved more mutations causing IV, some of them grouped within particular ethnicities, but all of them affecting the FLG gene. This corroborated the notion that FLG mutations are indeed the cause of IV. Moreover, scientists noted that this same gene is altered in some patients with atopic dermatitis, creating a major risk factor for the disease.\textsuperscript{125}
There is an abnormal micro and macro-architecture in the epidermis of patients with IV. On a microscopic level, the hallmark of IV is the reduction or complete loss of KhG, also known as hypogranulosis, because of the deficit in filaggrin. The disorganised KIF, unable to form bundles with filaggrin, show retraction towards the nucleus. This internal disarray also accounts for an abnormal packaging of LB, which show amorphous contents, and cannot properly arrange into lipid bilayers once extruded. Thus, the lack of filaggrin not only alters the architecture of the KCs, but also that of the extracellular lipids with a consequential effect on the skin barrier. Furthermore, the deficiency of amino acids from filaggrin degradation render IV skin with a higher pH than normal, this in turn decreases its antimicrobial and cohesive properties. The amino acids also form part of the natural moisturising factor of the skin, and their deficit is reflected in skin xerosis.

On a macroscopic level, IV skin shows very fine, translucent, and thin scales, that involve the extensor surfaces predominantly of the lower extremities (Figures 2, 12 and 13). The phenotype is more severe in winter because of low environmental moisture; the flexor surfaces which naturally have a higher humidity show mild or no involvement. The colour of the scales can be influenced by the natural skin colour of the affected individual, for instance dark-skinned patients may have a darker, "dirtier-looking" scale than lighter-skinned counterparts. Hyperlinearity of palms and soles is universally seen in patients with IV, and keratosis pilaris is a clinical sign in some patients (Figure 13).

Ichthyosis vulgaris can also be acquired and presents associated with a range of conditions including hormonal, chronic, and autoimmune diseases, or related to malignancy and medications. Associated conditions have been grouped in a table in Appendix 2. Clinically and histologically, acquired and inherited IV are identical, but they can be differentiated by age of onset and clinical course. While congenital IV appears in childhood and improves with age, acquired IV appears at a later age and may worsen along with the underlying condition.
Inherited IV can be associated with atopic dermatitis (AD), asthma, and hay fever because mutations in \textit{FLG} are also risk factors for these conditions.\textsuperscript{127,128} Indeed the odds ratio [OR] of loss of \textit{FLG} in one allele and its association with eczema is 3.12; [95\% CI: 2.57-3.79].\textsuperscript{128} The AD associated with IV presents early in life and is persistent.

Up to this date around 40 mutations\textsuperscript{129} in \textit{FLG} have been found linked to IV and/or atopic dermatitis; these mutations are population specific, meaning that Europeans harbour totally different mutations than Asians do.\textsuperscript{129} This makes it hard for scientists to develop a screening program for IV or AD since not all populations have been studied to find which part of the gene is altered. The link between asthma and \textit{FLG} mutations is not yet understood because \textit{FLG} is not expressed in the lower respiratory tract, but the OR of acquiring asthma are 1.48; [95\% CI, 1.32-1.66].\textsuperscript{128,129} However profilagrin is expressed in the outer layers of the oral and nasal mucosae, so this link must be studied further.\textsuperscript{129}

A study of European patients with \textit{FLG} mutations\textsuperscript{61} and AD showed a 22\% reduction in the concentration of UCA compared to individuals with a normal \textit{FLG} gene (p-value 0.0003).\textsuperscript{61} Though only one patient had ichthyosis vulgaris, it can be estimated that patients with IV will also lack this important UVB scavenger.\textsuperscript{116} It is also thought that perhaps the mutation of \textit{FLG} confers an advantageous absorption of sun rays for Vitamin
D synthesis in the skin of Northern Europeans. As UCA helps protect the integument from the deleterious effects of UVB, it can be inferred that patients with IV will be more sensitive to UVR. A Danish cohort study from 1977 to 2006 noticed an increase in non-melanoma skin cancer in patients with AD: a 1.41 risk for BCC [95% CI 1.07, 1.83] and 2.48 risk for SCC [95% CI 1.00, 5.11]. Thus, a study which relates skin cancer with filaggrin mutations and/or ichthyosis vulgaris is warranted.

Patients with IV have an abnormal barrier. The skin of these patients has a very high metabolic activity derived from the need to compensate for this barrier loss. Though not proven with ichthyosis vulgaris, it has been noted that patients with ichthyosis and failure to thrive need two to ten times more calories than patients with failure to thrive but without this skin condition. Patients with partial or total loss of FLG also have more energetic requirements derived from the up-regulation of inflammation markers that unsuccessfully try to compensate for the abnormal barrier (Figures 6 and 14). A study by Winge and colleagues analysed changes in gene expression in patients with AD or IV who were heterozygous or homozygous for FLG mutations, and compared them to normal-filaggrin individuals. The group noticed that the less filaggrin patients had, the more their skin expressed compensatory markers (Figure 14), including calcium regulation, adhesion markers, lipid transporters, and others. These results show that the epidermis in patients with reduced or no filaggrin must attempt to recover for this loss by increasing cellular proliferation and differentiation and by amplifying the synthesis of lipids and adhesion markers in a failed attempt to regenerate the epidermis.

![Figure 14. Changes in gene expression according to filaggrin mutations. From Winge et al.](image)

<table>
<thead>
<tr>
<th>Phenotype and genotype</th>
<th>Upregulated</th>
<th>Downregulated</th>
</tr>
</thead>
<tbody>
<tr>
<td>AD FLG+/+</td>
<td>131</td>
<td>181</td>
</tr>
<tr>
<td>AD FLG+/−</td>
<td>328</td>
<td>429</td>
</tr>
<tr>
<td>AD/IV FLG−/−</td>
<td>1833</td>
<td>1466</td>
</tr>
<tr>
<td>Total # genes</td>
<td>2292</td>
<td>2076</td>
</tr>
</tbody>
</table>

Genes with a minimum two-fold change and p-values<0.0005 were included. Top up- and down-regulated genes for included patients depending on FLG genotype. Genes with minimum 2-fold change and p-value<0.0005 were included.
3.3 ROLE OF STEROID SULPHATASE IN EPIDERMAL DIFFERENTIATION AND ITS LINK TO ICHTHYOSIS

X-linked ichthyosis is the second most common within the family of ichthyoses; it affects from 1 in 2000 to 1 in 6000 males according to Wells and Kerr.\textsuperscript{133,134} It is believed that XLI (OMIM # 308100) occurs at the same frequency in all races,\textsuperscript{119} however a report from Spain in 1977 suggests that the condition may be more common in certain ethnic groups.\textsuperscript{135} In 2010, Craig and colleagues\textsuperscript{136} found different incidences of XLI depending on ethnicity (not statistically significant) and a total incidence of XLI in 1:1500 males in California. Craig’s study also reviewed six studies of four different countries’ XLI prevalence; it seems that this prevalence is higher than reported\textsuperscript{117} and averages about in 1:1510 pregnancies of male babies worldwide (95% CI 1:1360–1:1670).\textsuperscript{136}

In 1966, Wells and Kerr\textsuperscript{117} compared IV and XLI. By gathering a group of 375 individuals who lived with ichthyosis, they were able to recognise that XLI had a more severe course than IV, although the report does not present any statistical values. On their retrospective analysis they determined that XLI affected 84% of patients within three months of birth, whereas IV affected only 40% (no p-value).\textsuperscript{117} They also noted that, compared to IV, XLI had more scaling of the sides of the face, neck, and flexures with darker-coloured, larger, and adherent scales (Figures 2, 12, 13 and 17). Though patients with IV improved significantly with age, patients with XLI sometimes worsened.\textsuperscript{117}

XLI occurs because of a deletion in the steroid sulphatase gene located in the short arm of the X chromosome, on Xp22.3 (Figure 15).\textsuperscript{137} STS is found everywhere in the body in varying quantities,\textsuperscript{138} the highest concentration is in the human placenta where it creates oestriol from steroid precursors.\textsuperscript{139} Large amounts of STS are found in the liver and the adrenal glands where it desulphates reproductive hormones. This process is important in normal tissues and increases in malignancies such as breast and ovarian cancer.\textsuperscript{138,140} STS is thought to also have a role in brain function, specifically memory, as well as a role in the immune system and in the production of skin androgens.\textsuperscript{138}
Figure 15. Short arm of X chromosome showing different possible mutations. Adapted from Paige et al.\textsuperscript{137}

Regarding the skin, CS regulates synthesis of TG, FLG, involucrin, and other important elements in the nucleated epidermis (Figure 11 in blue). Patients with XLI do not have a problem with this aspect, because they produce normal cholesterol sulphate, however, their CS does not undergo the normal desulphatation necessary for adequate protease activity, pH regulation, lamellar bilayer organisation, and cholesterol synthesis (Figure 11 in purple).

Cholesterol sulphate accumulates excessively in XLI; in fact these patients have five times more CS in the stratum corneum (p<0.0025)\textsuperscript{141} comprising nearly 12% of the lipid weight, whilst it would normally be less than 1%.\textsuperscript{106} The surplus has a detrimental effect in the homeostasis of the epidermis (Figure 16). Firstly, the excess of CS inhibits the proteases that should cleave corneodesmosomes for desquamation,\textsuperscript{106} consequently, the squames do not shed, and the skin becomes hyperkeratotic. Secondly, CS is more acidic than when desulphated, this causes an extreme low pH in the patients’ skin which can also inhibit KLK action.\textsuperscript{84} Thirdly, CS cannot intermix properly with the other lipids in the lamellae,\textsuperscript{84} causing disorganised bilayers and increased trans-epidermal water loss (TEWL).\textsuperscript{142} Finally, the extra CS leads to a negative feedback inhibiting cholesterol synthesis in the lower epidermis,\textsuperscript{141} which contributes to xerotic skin. A summary of some epidermal changes in XLI patients is seen in figure 16.\textsuperscript{143}
Figure 16. Clinical manifestations of XLI. Adapted from Lai-Cheong et al.143

Hoppe and colleagues144 studied the epidermis of 14 patients with XLI and 14 healthy volunteers. They noticed that XLI patients' skin had almost twice as much TEWL ($p = 0.0038$) reflecting an abnormal barrier function of their skin compared to counterparts without the mutation.144 This could be due to the disorganised bilayers formed when CS mixes with other SC lipids.106,142 This study did not find significant differences in surface pH values regarding XLI patients [5.02 ± 0.63] versus controls [4.62 ± 0.39] ($p = 0.064$).144 So perhaps the pH alteration may not be as important as direct CS inhibition for protease activity.

The hallmark microscopic image of XLI is hyperkeratosis and, in contrast to IV, the granular layer is normal or prominent. At the macroscopic level, the phenotype of XLI generally appears during the first few weeks145 after birth with shedding84,119 of loose, light-coloured scales over the entire body. These scales keep increasing and become darker, more adherent and plate-like as the child gets older (Figures 12 and 17).145 The scales are more prominent on the back and sides of the neck and the lower abdomen119 but involve all extensor surfaces especially those of the lower extremities. Flexor surfaces and the face may be mildly involved or spared (Figures 2, 12, 17). These patients do not present the hyperlinearity of IV,119 or involvement of nails and hair.146,147

Non-cutaneous findings of XLI include asymptomatic flour-like opacities of the cornea,146 which affect from 10 to 50% of patients.148,149 The sexual development and fertility of XLI males have been found to be normal146,150 but cryptorchidism and testicular
cancer have been described.\textsuperscript{151} Although, Elias et al.\textsuperscript{84} suggest this could be due to contiguous-gene syndromes.

![Image of clinical appearance of XLI](image)

Figure 17. Clinical appearance of XLI. Adapted from Fernandes et al.\textsuperscript{146} and Hernandez-Martin et al.\textsuperscript{147}

Though 90\% of XLI patients have a complete deletion of the STS gene,\textsuperscript{152} other mutations with loss of function have been described.\textsuperscript{153-155} Larger deletions have also occurred making XLI part of a syndrome.\textsuperscript{137,156,157} As seen in figure 15, neighbouring areas of STS encode genes related to other medical conditions such as Kallman syndrome, mental retardation, and chondrodysplasia punctata. All these are included in the current classification of ichthyoses\textsuperscript{20} considered as contiguous gene syndromes. As well as these syndromes, XLI has also been associated with cognitive abnormalities.\textsuperscript{137} In 2008, Kent et al.\textsuperscript{158} reviewed 25 children with XLI to determine the risk of attention deficit hyperactivity disorder (ADHD) and autism spectrum disorders. A surprising 40\% of these children met Diagnostic and Statistical Manual of Mental Disorders, 4th revision (DSM-V-IV) criteria and fulfilled a validated scoring system for ADHD that takes into account sex and age of patients. Moreover, the group noticed that large deletions of the X chromosome,
probably part of contiguous gene syndromes, were related to autistic disorders in five of the children (no statistical value provided).\textsuperscript{158}

STS deficiency is diagnosed in a routine screen during pregnancy where mothers show diminished oestriol levels.\textsuperscript{84,136,158} STS deficiency in the placenta is associated with a faulty labour because of little cervical dilatation;\textsuperscript{158} this occurs in around 30\% of XLI patients.\textsuperscript{21,158} The advantage of prenatal diagnosis has allowed physicians to recognise more cases of XLI than in the previous years, therefore showing increased prevalence.\textsuperscript{136}

Once the baby is born, the deficiency is confirmed by a blood test that analyses STS activity in leukocytes.

A patient diagnosed with XLI, should be referred for consultation with an ophthalmologist to check for eye abnormalities. Also, physicians caring for these patients must also consider a possible risk of testicular maldescent and cancer, as well as cognitive-behavioural problems and syndromes associated with these conditions. Physicians must also advise mothers about risk of failure of labour in future pregnancies.\textsuperscript{159}

Over the years, XLI has been treated with keratolytics and emollients but, thus far, there are no studies that attempt adding the absent STS into their integument. The treatments of XLI and IV will be discussed in the next chapter.

Table 2 shows a comparison of IV and XLI including scale characteristics, associated clinical features, associated diseases and histology. The previous paragraphs and table 2 underline the fact that these conditions are completely different in cause and physiopathology, as well as appearing different microscopically and macroscopically. Therefore therapy to these conditions should also be approached individually.
<table>
<thead>
<tr>
<th></th>
<th><strong>ICHTHYOSIS VULGARIS</strong></th>
<th><strong>X-LINKED ICHTHYOSIS</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>MUTATION</strong></td>
<td>FLG, chromosome 1q21</td>
<td>STS, chromosome Xp22.3</td>
</tr>
<tr>
<td><strong>INHERITANCE</strong></td>
<td>Autosomal Semi-dominant</td>
<td>X-linked Recessive</td>
</tr>
<tr>
<td><strong>EPIDEMIOLOGY</strong></td>
<td>1 in 250 to 1 in 1,000</td>
<td>1 in 1,500 to 1 in 6,000</td>
</tr>
<tr>
<td><strong>DEFECT</strong></td>
<td>Reduction of natural</td>
<td>Abnormal desquamation</td>
</tr>
<tr>
<td></td>
<td>moisturising factor</td>
<td>due to excess of CS</td>
</tr>
<tr>
<td></td>
<td>Abnormal skin barrier</td>
<td>Decreased protease</td>
</tr>
<tr>
<td></td>
<td>Increased skin pH</td>
<td>activity Decreased SC</td>
</tr>
<tr>
<td></td>
<td></td>
<td>pH</td>
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<tr>
<td><strong>SCALE CHARACTERISTICS</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Fine</td>
<td>Thicker than IV</td>
</tr>
<tr>
<td></td>
<td>Small</td>
<td>Smaller and in greater</td>
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<tr>
<td></td>
<td>Translucent</td>
<td>number than IV</td>
</tr>
<tr>
<td></td>
<td>Rounder edges</td>
<td>Dark-coloured</td>
</tr>
<tr>
<td></td>
<td>Bran, flaky texture</td>
<td>Polygonal</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Adhered to skin</td>
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<tr>
<td><strong>OTHER CLINICAL FEATURES</strong></td>
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<tr>
<td></td>
<td>No clinical phenotype</td>
<td>Initially manifests</td>
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<td></td>
<td>at birth, but develops</td>
<td>within first weeks of</td>
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<td></td>
<td>within the first</td>
<td>birth</td>
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<td></td>
<td>year of life.</td>
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<tr>
<td></td>
<td>It stabilises in</td>
<td>It stabilises in</td>
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<td></td>
<td>adolescence and</td>
<td>adolescence, but</td>
</tr>
<tr>
<td></td>
<td>improves with age</td>
<td>doesn't keep improving</td>
</tr>
<tr>
<td></td>
<td>No maternal labour</td>
<td>Prolonged maternal</td>
</tr>
<tr>
<td></td>
<td>difficulties</td>
<td>labour</td>
</tr>
<tr>
<td></td>
<td>No reproductive organ</td>
<td>Cryptorchidism?</td>
</tr>
<tr>
<td></td>
<td>alteration</td>
<td>Delayed puberty?</td>
</tr>
<tr>
<td></td>
<td>Improvement in summer</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Palmoplantar hyperlinearity</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Keratosis pilaris</td>
<td>Asymptomatic corneal</td>
</tr>
<tr>
<td></td>
<td></td>
<td>opacities</td>
</tr>
<tr>
<td><strong>ASSOCIATED CONDITIONS</strong></td>
<td></td>
<td>Atopic Dermatitis</td>
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<tr>
<td></td>
<td></td>
<td>Allergic rhino</td>
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<tr>
<td></td>
<td></td>
<td>conjunctivitis</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Non-melanoma skin</td>
</tr>
<tr>
<td></td>
<td></td>
<td>cancer?</td>
</tr>
<tr>
<td><strong>BIOPSY</strong></td>
<td>Hypogranulosis</td>
<td>Normal SG or hypergranalnosis</td>
</tr>
<tr>
<td></td>
<td>Reduction or absence of</td>
<td>Normal Kg</td>
</tr>
<tr>
<td></td>
<td>KhG</td>
<td>Marked hyperkeratosis</td>
</tr>
</tbody>
</table>

Table 2. Comparison of Ichthyosis Vulgaris and X-linked Ichthyosis
3.4 A HOLISTIC VIEW OF THE COMMON ICHTHYOSES

Despite the fact that the common ichthyoses mainly affect the skin, it is impossible to disregard the effect of these conditions on the patients' quality of life (QoL). There seems to be a general perception of IV and XLI as "mild" diseases in the medical community.\textsuperscript{159,160} Although comparing them to other congenital ichthyoses (CI) this is true, the severity of XLI and IV varies greatly from patient to patient and may even be worsened by associated illnesses. In addition, regardless of severity, patients have to endure this condition their entire life. The common ichthyoses appear shortly after birth and, consequently, have an impact on the child, carer, and on the family as a whole. Because the ichthyoses are chronic, incurable conditions, they will be an issue in the everyday life of patients and will shape choices including clothes, daily activities, career and hobbies.\textsuperscript{161}

In 2003, Ganemo \textit{et al.}\textsuperscript{162} assessed 10 ichthyosis patients with the Nottingham Health Profile (NHP) and with a face-to-face structured interview. The NHP is a questionnaire filled out by patients which provides a subjective view of their condition on emotional, social, and physical realms. The interview consisted of 21 open-ended questions regarding thoughts about the ichthyosis, how it has shaped major decisions in their lives and other aspects of childhood and adolescence.\textsuperscript{162} Though none of the patients had XLI or IV and the study presents several selection and publication biases, important conclusions can be gathered about the effect of the condition in patients' lives. For instance, all patients reported that during childhood their parents had a deep involvement in caring for their illness. Indeed the study reflects how time-consuming ichthyosis is: a bath can last three to four hours, the skin must be moisturised at least twice a day to prevent painful fissures and cracks due to xerosis. If the condition is hyperproliferative, the house must be cleaned more often than normal because the excessive shedding of scales contributes to dust. The lipids in the emollients accumulate on clothes and washers, which need replacement more often than normal.\textsuperscript{162} All of these contribute to the burden of the disease in the family.

In Ganemo's description,\textsuperscript{162} all patients (10/10) reported to have been bullied by peers even as adults. Most patients (7/10) also reported that their condition had been an important factor when deciding on a career path (7/10) and when choosing to have a
family (6/10). All patients (10/10) reported that childhood was the most troublesome time considering the condition. This is consistent with the fact that both IV and XLI present with more severe phenotypes during childhood. Moreover, during childhood patients learn to care for themselves as well as to modify their everyday life considering their ichthyosis and its implications.

In 2011, Kamalpour and colleagues\textsuperscript{160} gathered the largest cohort to date, 235 patients with ichthyoses including 31 subjects with IV and 29 with XLI. They analysed the patients' QoL and their usage of resources defined as a) time spent in treating the skin condition every day, and b) the average number of visits to dermatology departments per year.\textsuperscript{160} Despite the fact that the study has a clear selection bias, its conclusions were statistically significant and portray how a lower QoL is directly correlated with severity of hyperkeratosis ($\beta = 0.27$, $p < 0.01$) and of erythema ($\beta = 0.27$, $p < 0.01$).\textsuperscript{160} Time spent to treat the condition by patients or their caregivers, the type of ichthyosis, and a lower patient age also negatively affect the QoL.

The study shows as well that the younger the patient is, the more resources he or she utilises.\textsuperscript{160} Younger patients or their carers needed more time to treat the condition compared to older individuals. Patients with XLI and IV spend an average of 16 to 30 minutes per day treating their skin disease.\textsuperscript{160} Younger patients also make more yearly visits to dermatologists. What is more, the disease also has a deep financial impact on families; Styperek and colleagues\textsuperscript{163} reported, in the United States, an average annual societal expenditure of $3,192 and a personal out-of-pocket cost of $1,182 for each patient with ichthyosis.\textsuperscript{163} The societal expenditure corresponds to the funds from tax payers which contribute to healthcare resources associated to treating patients with ichthyoses; out of pocket costs corresponds to the amount of money patients have to spend on their own on moisturisers, fuel to attend outpatient visits and funds lost when missing days of work.\textsuperscript{163}

As a general conclusion, Kamalpour \textit{et al.}\textsuperscript{160} noted that a decrease in the use of resources would be seen if more and better therapies were available for the patients as well as if both patient and family were educated thoroughly upon diagnosis. This highlights the importance of support groups in a) the development of children with ichthyosis and b) the acquisition of experience on how to deal with the condition from
fellow patients and families. In fact the study found out that families with a previous history of the condition seemed to utilise fewer resources.

In 2013, Dreyfus et al.\textsuperscript{164} developed a targeted quality of life scale for the ichthyoses (IQoL) that takes into account 32 items to grade how impairing the condition is for the patient. They then compared it to two QoL related questionnaires: the Dermatology Life Quality Index (DLQI) and the Short Form-12 Health-related questionnaire (SF-12). In both the IQoL and the DLQI, a higher score means a severe impairment in the quality of life, whereas in the SF-12 a higher score signifies better QoL.

The study showed that more severe disease correlated with higher scores for the IQoL; the IQoL scores directly correlated with the DLQI (p<0.001) and inversely with the SF-12 (p<0.001).\textsuperscript{164} Moreover the IQoL took into account items specifically relevant to the ichthyoses such as eye xerosis and ear obstruction due to the scales, as well as skin pain or discomfort due to fissures. Yet this tool is more cumbersome than both DLQI and SF-12 as patients need to rate 32 items rather than 10 or 12 respectively.\textsuperscript{164}

Selection biases were found on the IQoL study as it included mostly women and only took into account participants who agreed to volunteer the information (62 out of 130), thus it may not include a good sample of the ichthyoses population. Attrition and reporting bias was also found; three questionnaires were incorrectly filled out and rated as invalid; 59 patients were accounted for on the IQoL, 58 patients for DLQI and 53 patients for SF-12.\textsuperscript{164}

However, the study shows that QoL has different results when disease-specific signs or symptoms are accounted for. For instance, 19 patients appeared to have a severe QoL reduction according to the IQoL and only 15 patients fell in the same category with the DLQI.\textsuperscript{164} The fact that the IQoL presented a good correlation to a global assessment of disease severity shows that this could be a good tool to use upon first consultation of a newly diagnosed patient as well as a regular control during childhood, adolescence, and adulthood. Furthermore, the fact that this scale takes into account disease-specific items, could open a door for doctor-patient discussion and education upon newly diagnosed individuals. For example, the family of a newly diagnosed baby with IV may not be aware that the scales could cause ear obstruction and impair hearing, which they would see as one of the items listed in the questionnaire. The same rationale could apply to eye xerosis, changes to daily leisure and holiday activities including caring for the home,
environmental temperature of vacation sites and others. A copy of the IQoL can be found on Appendix 3.
3.5 TREATMENT OF THE COMMON ICHTHYoses

Dermatological management of ichthyosis vulgaris and X-linked ichthyosis is similar. No current treatment for the congenital ichthyoses targets the altered pathway. IV is caused by the deficit of a protein, while XLI is caused by excess of a lipid derivative, yet they are treated with the same topical and oral medications. General measures aim to maintain a humid environment to decrease the xerosis, baths with soap substitutes are important but care must be taken as these can make the bath slippery. Sometimes patients, especially with XLI, will scrub with sponges to reduce the scales but, vigorous use of brushes while scrubbing can irritate the skin. Moisturisers should be applied to damp skin within three minutes of showering to hold water in.

Current trials of the available ichthyoses treatments seem to be biased and gather small cohorts. In 2013, Hernandez-Martin et al. reviewed, systematically, six randomised controlled trials (RCT) for the congenital ichthyoses excluding IV. The group observed selection bias because most of the trials had small numbers of patients, randomisation was unclear and allocation to treatment or placebo was not well defined. The group also noticed performance bias in four of the six studies because the blinding was not adequately established and some patients had been lost to follow up. Three of the six RCTs also presented reporting bias. This systematic review observed an important need for adequate evaluation of current therapies for ichthyoses, including larger samples, multicentre trials, appropriate randomised allocation, blinding, and accountability of lost patients or use of intention-to-treat analysis.

3.5.1 MOISTURISERS

Moisturisers are products that can add or hold water in the SC as well as prevent TEWL. Humectants such as urea and glycerine are hygroscopic (attract and hold water). Lipids, such as ceramides, cholesterol and free-fatty acids, incorporate into the bilayers and restore barrier function. Regarding formulations, lotions contain more water than creams, creams more water than ointments. Ointments are greasier than creams and creams are greasier than gels and lotions. Greasier products can hold water much better but are generally not cosmetically accepted, whereas the more watery products easily evaporate, cannot prevent much TEWL but they are better accepted by patients.
Moisturisers also aim to restore the skin barrier and decrease xerosis in both XLI and IV. There are no RCTs that compare moisturisers in patients with ichthyosis, and the topical treatments are many times seen as cosmetics instead of therapy, yet their importance is highlighted by the decreased need of steroids in patients with AD who adequately moisturise their skin. Patient preference must be accounted for when choosing a moisturiser, as well as lifestyle, possible allergies, and product consistency. Though ointments are great moisture retainers, they make the skin greasy and shiny, its thickness carries a higher risk of follicular obstruction, and they are not usually suitable for the face.

In 1983, Lykkesfeldt et al. published the first clinical trial using cholesterol as a treatment for XLI and comparing it to urea. The study was not randomised, there was no blinding of subjects or evaluators and there is no clear indication of the study’s protocol. This was a half-side trial with one side using a topical formulation with 10% cholesterol, and the other side with 10% urea. The results mention an excellent response to both topical treatments with an advantage of the cholesterol-based over urea-based therapy in 13 patients ($p<0.001$) founded only on clinical criteria.

Zettersten and colleagues, performed a study on barrier recovery time in XLI versus controls and then barrier recovery in XLI treated with cholesterol versus vehicle. The group first compared barrier recovery in XLI patients versus controls after tape stripping. The results are in figure 18A where patients with XLI do not recover the barrier as well as controls at 6 hours ($p<0.02$); and at 24 hours after tape stripping ($p=0.008$). After this the group applied either cholesterol or vehicle to XLI skin. They noticed better barrier recovery times in those treated with cholesterol as can be seen in figure 18B at 6 hours ($p=0.001$) and at 24 hours ($p=0.037$). However, the recovery is not perfect and does not match that of normal subjects at the same time points (Figure 18 red, blue and purple lines). Zettersten et al. were very thorough when testing for barrier abnormalities. However, the major complaint of patients with XLI is hyperkeratosis. Indeed, in practice, patients with XLI are treated with moisturisers containing lactic acid, urea or glycerol in a similar fashion as IV patients are treated.
Tadini et al.\textsuperscript{173} gathered 30 patients with IV to perform a half-side comparison between a 10% urea-based lotion and a glycerol-based cream on an evaluator-blinded, RCT. They treated the patients for four weeks and an evaluator used a 5-point scale that measured the hyperkeratosis, erythema and fissures.\textsuperscript{173} The scores decreased significantly ($p = 0.0001$) from a score of 9.5 to 3.3 with the urea-based formulation and from 9.5 to 5.7 after the glycerol-based medication. The study mentions few limitations, however, there is no clear impression of the protocol, methodology, type of randomisation used or even sites where the medication was applied. Three patients dropped out of the study because of irritation and it is unknown whether they were accounted for in the results.

Some moisturising formulations can also be keratolytic, some can contain salicylic acid, alpha-hydroxy acids, and others. Physicians must always take into account the faulty skin barrier in patients with XLI and IV which increases absorption and therefore toxicity of medication. For this reason salicylic acid should be used to treat less than 20% of the body surface area in children and this, along with urea, should be avoided during the first year of life.\textsuperscript{21}

**3.5.2 TOPICAL THERAPY**

The current topical medications to treat ichthyoses include vitamin D analogs and retinoids. Topical calcipotriol has been evaluated for the ichthyoses in two double-blinded, randomised, vehicle-controlled trials.\textsuperscript{174,175} The first study, performed by Lucker
and colleagues,\textsuperscript{175} included only six patients, none of them with XLI or IV; still, the research found an advantage of calcipotriol over vehicle (no statistical value provided). Kragballe and colleagues,\textsuperscript{174} gathered 67 patients, including nine with IV and eight with XLI. They used calcipotriol for 12 weeks with a maximum weekly allowance of 120g. 18 of the 67 patients developed skin irritation (p<0.01). A 6-point scale, used for treatment response, showed a marked improvement in the side treated with calcipotriol in 50% of the patients with XLI evaluated by investigators (p=0.03) and by patients themselves (p=0.03).\textsuperscript{169,174} Though calcipotriol is a good option, it can only be used for short-term therapy in small areas with a maximum weekly dosage of 100g according to the British national formulary to avoid the important risk of hypercalcaemia.\textsuperscript{174,175}

Topical therapy with tazarotene has been proven to modulate keratinocyte proliferation and differentiation\textsuperscript{176} and has advantages over other more irritant retinoids, such as tretinoin and 13-cis-retinoic acid.\textsuperscript{177} In 1999, Hofmann and colleagues\textsuperscript{177} used tazarotene gel 0.05\% as a treatment for several congenital ichthyoses, including four XLI patients. The study was open-label, half-side, controlled by the patient and compared with a 10\% urea-containing ointment in the other arm. One of the four patients with XLI showed mild irritancy with the treatment but not enough to discontinue therapy. According to the results and, as seen in the image below (figure 19), three patients had excellent and one had good reduction of scales and skin roughness (no statistical value provided). The study also noticed residual therapy of the tazarotene-treated side for up to eight weeks once treatment ended.\textsuperscript{177,178} This study lacked a good methodology, it was not randomised and neither evaluators nor patients were blinded. Although XLI patients showed good clinical effect, the scale used was arbitrary. Moreover, the study only included four patients with XLI.
Trials of 1α-hydroxyvitamin D₃, 1α,24-dihydroxyvitamin D₃, 13-cis-retinoic acid, among others have been attempted for the ichthyoses but the results showed inefficacy in the case of the vitamin D derivatives or marked irritation that warranted withdrawal in the case of the retinoic acid.

3.5.3 ORAL MEDICATIONS

Up to this date, retinoids are the only oral therapy approved to treat ichthyoses and these medications have their own range of side effects including hepatotoxicity and hyperostosis. Despite the harsh adverse effects, retinoids have been used in children with severe disease as they are the only available therapy for recalcitrant cases.

Acitretin was evaluated as a treatment for patients with XLI in an open-label trial in 1988. The other options at the time were etretinate and isotretinoin. Etretinate has been withdrawn from the market because of the severe risk of birth defects as the medication is stored in the body for a long time. It has been substituted by acitretin which is one of etretinate's metabolites. Isotretinoin is another oral retinoid available on the market but it is currently only licensed to treat acne.
The study of acitretin\textsuperscript{182} was performed for four months in eight patients. The study does not specify how many evaluators saw the patients or if the same evaluator was used during subsequential visits. Moreover, the evaluators were not blinded and no control, such as a placebo group, was used. Yet, with acitretin as a treatment, three of the patients achieved complete remission, three achieved good control and improvement of the ichthyotic skin and two noticed mild improvements (no statistical value provided). Furthermore, the improvements persisted for four to six weeks after stopping the treatment. One of the patients discontinued treatment due to severe pruritus after eight weeks.\textsuperscript{182}

A small trial of alitretinoin, currently licensed for hand eczema, was performed on four patients with ichthyosis, none of them with IV or XLI.\textsuperscript{183} The results had a low power and even showed hypothyroidism as a side effect in two of the four patients. The author suggests that alitretinoin may be considered in those women of childbearing age who may want a therapy with less lag-time before becoming pregnant. Yet, the risk of causing hypothyroidism in a patient already affected by another disease should be considered.

A new therapy evaluated is liarozole\textsuperscript{169} which is an inhibitor of the retinoid pathway and is available for trials in topical and oral forms. The pharmaceutical company claims a shorter time of teratogenicity risk as the main advantage of this drug. However, liarozole is still on Phase II/III trials and the studies thus far have low power, indicating that there would be no real advantage of selecting it over an oral retinoid such as acitretin.\textsuperscript{169}

In a review of retinoid therapy for children,\textsuperscript{184} it is mentioned that the best effect of this medication is in the hyperkeratosis of the patients' skin. Yet, the side effects become a limitation to its use, especially in the paediatric sphere. The review noted that the most common side effect in children is xerosis of all mucosae, similarly to adults. Skeletal effects include calcification of tendons and ligaments, hyperostosis and premature epiphyseal closure.\textsuperscript{184} Therefore the lowest possible dose should be used to prevent these abnormalities. With care and close observation, the risk of these side effects can be minimised; indeed, an appropriate therapy can have great benefit which was seen in a small group of patients who, while using retinoids, improved their height and weight.\textsuperscript{184} Because IV has an up-regulation of over 2000 genes to attempt the restoration of the epidermal barrier,\textsuperscript{131} it is a reasonable thought that the energy
requirements and, therefore, caloric intake must be higher than that of normal subjects\textsuperscript{132} or even of patients with XLI where there is no significant up-regulation of epidermal differentiation genes.\textsuperscript{144}

Thus far, the management of the ichthyoses remains empirical and is based on opinion of experts\textsuperscript{21,22,91,166,167} who have devoted a significant amount of time to research in the area. Each of the experts suggest bath alternatives, one or another topical moisturiser, and oral retinoid therapy for severe cases.
Chapter 4

THE PROPOSED STUDY
Chapter 4:

4.1 PROPOSED STUDY

The Use of Steroid Sulphatase Delivered in Liposomes as a Novel Therapy for X-Linked Ichthyosis

4.2 ABSTRACT

X-linked ichthyosis is a skin condition caused by the absence of steroid sulphatase, an enzyme that converts cholesterol sulphate to cholesterol in the upper stratum granulosum and lower stratum corneum. The disease is currently treated with moisturisers, keratolytics and retinoids in the same way as all other ichthyoses. No previous treatment has attempted delivery of the absent enzyme into its site of action. This study proposes a novel therapy for X-linked ichthyosis with steroid sulphatase carried inside liposomes, a lipid-miscible vehicle. The treatment will be done for 14 days on a humanised-skin model and it will be controlled with vehicle and tazarotene 0.05%. Evaluation of results will be performed objectively by measuring TEWL, pH, immunohistochemistry and with a record of clinical images. A blinded evaluator will assess the grafts with a 4-point hyperkeratosis scale. Serum measurements of cholesterol sulphate will be taken at baseline and at the end of the experiment to assess effects on systemic cholesterol sulphate levels. It is hoped that the approach of a causative therapy for XLI will signify in an effective medication, decreased application times, increased compliance, and resolution of phenotype, with a consequent improvement of quality of life for patients and families.

Abbreviations Used

XLI, X-linked ichthyosis; STS, steroid sulphatase; CS, cholesterol sulphate; SC, stratum corneum; QoL, quality of life; kDa, kilo Daltons; SG, stratum granulosum; DHEAS: dehydroepiandrosterone sulphate; cDNA, complementary DNA; pSTS, steroid sulphatase protein expression vector; rhSTS, recombinant human steroid sulphatase; TEWL, trans epidermal water loss.
4.3 INTRODUCTION

Recessive X-linked ichthyosis (XLI) is a congenital disorder of cornification in which the individual keratinocytes do not shed properly. XLI is caused by a deletion in the X chromosome at Xp22.3, the locus for steroid sulphatase (STS). This enzyme has many roles in the human but mainly removes sulphate groups in the conversion of hormones. In the skin, steroid sulphatase converts cholesterol sulphate (CS) into cholesterol. Patients with XLI do not have STS and thus accumulate an excess of CS in their epidermis. This excess causes an abnormal lipid barrier but, more importantly, it inhibits the enzymes responsible for degrading the corneodesmosomes, which are the intercellular junctions in the stratum corneum (SC). Thus the cells do not desquamate resulting in a hyperkeratotic appearance.

Figure 20. Clinical image of X-linked ichthyosis. Adapted from Oji et al. X-linked ichthyosis usually appears in the first weeks of life with shedding of small, translucent scales over the entire body (Figure 20B). The scales slowly build up in the stratum corneum and turn darker, larger, thicker, and more adherent (Figure 20A). The condition affects mostly the sides of the face and neck, the lower abdomen, and the extensor surfaces, especially that of the lower extremities.

X-linked ichthyosis stabilises in puberty but will affect the individual for his entire life. This disease normally only occurs in males who acquire the faulty X chromosome from their mothers. Females with this mutation still have functioning STS from their other X chromosome, so the only way to acquire the condition is if the father has XLI and the mother is a carrier of the same mutation which is very rare but has been described in the
literature. XLI has associated signs such as corneal opacities, hypohydrosis, cryptorchidism and delayed puberty. It can also form part of syndromes when the deletion includes adjacent parts of the X chromosome. Consequentially XLI is associated with Kallman syndrome, mental retardation, chondrodysplasia punctata, short stature among others. All these diseases are caused by partial deletions of the X-chromosome and are categorised as contiguous gene syndromes.

Currently the diagnosis of XLI is prenatal with a regular screening for Down's syndrome and other aneuploidies around the twelfth week of pregnancy where low oestriol levels are detected. Normally, STS desulphates precursors into this hormone. The low oestriol levels can also cause a failure of labour in the third trimester. When the baby is born, the diagnosis is usually confirmed by a blood test that shows elevated CS levels.

Quality of life in patients with X-linked ichthyosis is affected, especially in patients with severe hyperkeratosis. The number of dermatology visits as well as time and money spent to treat XLI is substantial, especially during childhood when the disease is worse and affects the whole family. It is estimated that the average annual cost of a patient with congenital ichthyoses is $3,192 societal cost and $1182 out-of-pocket cost in the United States.

Treatment for XLI remains challenging. Currently, none of the available medications target the excess of CS. In 1983, Lykkesfeldt's group used cholesterol to treat XLI showing good results. However, in practicality, patients opt for other moisturisers at least twice daily to keep the skin hydrated, as well as some sort of keratolytic in the form of acids, especially alpha-hydroxy-acids. Topical retinoids have also been pursued with some degree of success but these are expensive, cause irritation, are only used in the most problematic areas and must also be applied once or twice daily. Oral retinoids, like acitretin, are an alternative for severe cases but they come with a range of important side effects such as hepatotoxicity and skeletal abnormalities which limits their use in children.

Gene therapy has also been researched for XLI but the cost of creating and delivering genetically modified keratinocytes are expensive and time-consuming. The therapies have been carried out in labs and tested in mice but it is unlikely that they will reach the current market as XLI is not a fatal disease, it can be controlled with other
therapies and there is too much risk in altering the genome versus the benefit that the patients may have.

Recent research on the ichthyoses has targeted defective enzymes and pathways to provide an adequate, individualised therapy. The advantages of targeted therapies include efficacy of treatment, reduction of application to at least every-other day which greatly increases compliance, decrease of resource utilisation and improvement of patients' quality of life (QoL). For that reason, it is only natural that a treatment attempting to deliver the lost STS for XLI is sought.

This study proposes the use of steroid sulphotase to treat X-linked ichthyosis. Although STS is an ideal therapy, the viability presents several barriers. Firstly, the STS molecule weighs about 63 kilo Daltons (kDa) whereas only molecules smaller than 5 kDa can traverse the stratum corneum. The second difficulty is reaching the site of STS activity. Elias et al. demonstrated that STS exerts its action in the stratum granulosum (SG)/SC junction; therefore, the molecule does not only have to enter but also traverse the full thickness of the SC which is 10 to 20 µM to reach its target.

To overcome the obstacle of the high molecular weight of STS, this study intends to deliver the molecule via liposomes (Figure 21) which are small lipid structures that can intermix with other lipids, such as those of the epidermis and therefore traverse through them. In a study by Aufenvenne et al., which has stimulated the basis of this proposal, the liposomes were able to reach the stratum granulosum to exert its action.

Figure 21. Structure of a liposome with STS. Adapted from Aufenvenne et al.
4.4 OBJECTIVE

The primary aim of the study is to test the efficacy of STS delivered in liposomes as a treatment for XLI.

The secondary aim of the study is to evaluate systemic absorption of steroid sulphatase.

4.5 SUGGESTED STUDY DESIGN

This study is a preclinical trial in a humanised-skin mouse model of XLI. The therapy will be controlled with vehicle and tazarotene. The clinical evaluator will be blinded to which treatment has been applied to the mouse. A statistician will be involved to determine the number of mice needed. Three subjects who meet the inclusion criteria will be selected.

4.6 ETHICAL APPROVAL

This study will be conducted according to Declaration of Helsinki principles and will be sent for approval to the institutional review board of the University Hospital of Wales. All individuals enrolled will give their informed consent.

All animal studies will seek approval by the Animal Care and Ethics Committee, and all experimental procedures will be conducted according to British laws and regulations.

4.7 PATIENT CONSENT

Written consent will be obtained from each patient with XLI for the biopsies. Each patient will receive a hand-out with clear details of the study and how their skin will be used. The patient information leaflet and the consent form are listed on Appendix 4 and 5 respectively.

4.8 SUBJECTS

Based on the study by Aufenvenne et al., because single keratinocytes are required to produce a bioengineered epidermis and because this is a preclinical experiment, only three individuals with XLI will be recruited from a specialised outpatient clinic and must have clinical, histological, biochemical and genetic diagnosis of the condition.
4.8.1 BIOCHEMICAL ANALYSIS

The biochemical analysis will measure the STS activity of blood leukocytes using dehydroepiandrosterone sulphate (DHEAS) as a substrate. This is the technique used to diagnose XLI patients currently.

4.8.2 GENETIC TESTING

A blood sample will be obtained to extract DNA and sequence the short arm of the X chromosome and confirm that the deletion only affects the STS gene. This is performed because we cannot be certain if any other genes in the X chromosome participate in epidermal differentiation or homeostasis.

The FLG gene will also be assessed for the most common mutations in the European population (R501X, 2282del4). This will prevent including patients with ichthyosis vulgaris and X-linked ichthyosis which have been described in the literature.

4.8.3 BIOPSY

One punch biopsy (3-5mm) per patient will be taken under local anaesthesia. The biopsy will be taken from the left or right buttock.

4.8.4 RECRUITMENT

Patients with X linked ichthyosis will be recruited from the database at the University Hospital of Wales. Due to the fact that XLI is a common disorder of 1 in 1500 to 1 in 6000 males, reaching the target number should not present any difficulties.

4.8.5 INCLUSION CRITERIA

- Male patients
- Patients with ages between 18-35 years
- Patients of European ancestry so that the relevant profilaggrin mutations: R501X and 2282del4 can be assessed
- Patients with a deletion in Xp22.3 resulting in absent STS enzyme determined by genetic and biochemical analysis, and compatible with clinical and histological evaluation
- Patients must be willing and able to tolerate venipuncture for a blood sample
- Patients must be willing and able to tolerate a punch biopsy
- Patients must speak and read English or Welsh language
- Patients must be willing and able to sign and to give written consent
• Patients must not have been on any oral or topical therapy for the past six weeks
• Patients must not have received acitretin in the past 3 years

4.8.6 EXCLUSION CRITERIA
• Females
• Patients younger than 18 or older than 35
• Patients who are unable to undertake any part of the venipuncture or biopsy process: sign and provide written consent, undergo venepuncture, undergo a punch biopsy
• Patients who are not of European background as they may have different FLG mutations
• Patients who cannot read or write English or Welsh languages
• Patients whose condition forms part of a contiguous gene syndrome, or patients who have a concomitant filaggrin mutation
• Patients who have applied any form of topical therapy or ingested any oral medications in the last 6 weeks
• Patients who have received acitretin within the past 3 years
• Patients with a skin condition other than XLI
• Patients with physical or mental disabilities
• Patients who are currently enrolled in any other trial or study

4.9 ANIMALS

Nude (nu/nu, NMRI background) mice will be purchased from Charles River and will be housed individually in pathogen-free conditions at the appropriate laboratory animal facility

4.10 ENZYME

In order to obtain an active enzyme, we will use the method of Sugawara et al.198 In brief, human STS complementary DNA (cDNA) containing a full-length STS protein expression vector (pSTS) will be transfected into monkey kidney COS-1 cells, and cultured into the appropriate environment. The cells will be incubated for 48h and then lysed to obtain the purified recombinant human steroid sulphatase (rhSTS). This rhSTS is the treatment.
4.10.1 STS ENZYME ACTIVITY

Before applying it to the skin, we must test that the STS is active. The activity of the enzyme will be tested using DHEAS as a substrate, as previously described by Sugawara et al.\textsuperscript{199} Briefly, 100 pmol DHEAS will be incubated with STS at 37° C for 1 hour. After this, desulphated DHEAS will be extracted. One unit of activity (1UA) will be set as the amount of STS that desulphates 1 pmol DHEAS per hour.

4.10.2 LIPOsome PREPARATION

Liposomes are the vehicle for the delivery of our treatment. They will be prepared using a modified version of the technique used by Sauer et al.\textsuperscript{200} which uses phosphatidylcholine from dry egg and cholesterol (Figure 21).\textsuperscript{191,200} The cholesterol aids with miscibility in the lipid layers. A derivative of polyethylene glycol used in Sauer et al. that ensures cellular uptake will not be used because the activity of steroid sulphotase is relevant only in the intercellular space. The liposome preparation will be vortexed in a buffer solution containing recombinant STS. The suspension will be sonicated to obtain large unilamellar vesicles, and after that it will be extruded to obtain small unilamellar vesicles. The diameter of the vesicles will be determined with a particle sizer.

4.10.3 IMMUNOHISTOCHEMISTRY

Immunohistochemistry will be performed in a preliminary experiment to determine the localisation of the STS by using an STS-antibody. This will ensure that our treatment reaches the granular layer. Immunohistochemistry will be also used for evaluation of results.

4.11 BIOENGINEERED SKIN

Using the model from Garcia \textit{et al.}\textsuperscript{201} enzymatic digestion of skin biopsies will produce separate cell cultures of keratinocytes and fibroblasts from XLI patients. The dermis will be formed with fibroblasts and clotting-regulating bovine derivatives\textsuperscript{191} in 6-well plates, in the same fashion as Garcia \textit{et al.} indicate, and emulating a wound healing process. The keratinocytes (1x10\textsuperscript{5} to 5x10\textsuperscript{5} cells per well) will be seeded on the preformed dermis after a 48 hour incubation with 1.2 mM of CaCl\textsubscript{2} to induce differentiation.
Once the bioengineered epidermis and dermis are formed, the skin will be grafted under sterile conditions onto 6-week-old nude mice. The bioengineered skin is expected to be ready for application of the STS-liposomes in eight weeks after grafting. Aufenvenne et al.\textsuperscript{191} noticed that the graft resembled the human phenotype macro and microscopically at 4 to 6 weeks, including hyperkeratosis and epidermal hyperplasia.

4.11.1 IN VIVO TESTING

For \textit{in-vivo} testing the STS deficient epidermis will be treated with two different liposome preparations; preparation A will contain a liposomal solution with 10 ng rhSTS; and preparation B will contain 40 ng rhSTS. The amount of rhSTS in the different topical formulations has been gathered from the study by Aufenvenne et al.\textsuperscript{191} and it may need modifications once 1UA of rhSTS has been determined in this experimental study. Choosing to treat with different concentrations of rhSTS will serve to determine if there is a dosage-dependent effect which will further corroborate the efficacy of the formulation. Empty liposomes and tazarotene 0.05\% will serve for control. The empty liposomes will serve as a vehicle control, and tazarotene has been selected since currently retinoid therapy is one of the best available treatments for XLI.\textsuperscript{177}

All treatments will be applied once every other day with a cotton applicator, separate for each treatment and for each graft. The treatment will last 14 days.

4.12 STATISTICAL CONSIDERATIONS

A statistician will be involved to determine study sample, including the number of mice. The statistician will also participate in handling data analysis.

4.13 EVALUATION OF RESULTS

The evaluation of results are included in a flow chart on figure 22 where it details all steps to follow from selection of patients until result acquisition.

4.13.1 BIOCHEMICAL ANALYSIS OF CS

A blood sample will be taken at the beginning of the study as a baseline to determine the amount of cholesterol sulphate and of STS activity.
At the end of the study, another blood sample will be taken from the mice to determine levels of CS and STS activity and to compare them to those from the beginning. This helps in determining systemic absorption of the topical treatment.

4.13.2 SKIN SURFACE PH

A baseline skin surface, non-invasive pH measurement will be taken before treatment is instituted with pH measuring strips. Once treatment is started, skin surface pH will be evaluated non-invasively every fourth day, one day before the treatment is applied.

4.13.3 TRANS EPIDERMAL WATER LOSS

A baseline measurement of TEWL will be taken using the non-invasive device, Tewameter TM 300©. Once treatment is started, TEWL will be measured every fourth day, one day after measuring pH, on the day the treatment is applied and before its application.

4.13.4 CLINICAL EVALUATION

A blinded clinical evaluator will assess the grafts on day zero and once every seven days. An arbitrary 4-point scale will be used where 0= no scaling, 1= mild scaling, 2=moderate scaling, and 3=severe scaling.

4.13.5 CLINICAL IMAGES

Clinical pictures will be performed at baseline and on the same day as the clinical evaluation takes place and before the evaluator grades the humanised mouse models.

4.13.6 MICROSCOPY

Once the treatment has finished, grafts will be compared with microscopy and immunohistochemistry. Sections of the graft will be stained with haematoxylin and eosin to evaluate the general morphology of the epidermis.

4.13.7 IMMUNOHISTOCHEMISTRY

Some sections will be analysed with immunohistochemistry for corneodesmosin and STS. Measuring the corneodesmosin will serve to determine the number of corneodesmosomes remaining in the SC; XLI is a condition where the stratum corneum shows an increased number of corneodesmosomes. Thus it is expected that the
amount of corneodesmosomes will decrease with the novel treatment compared to the vehicle and the tazarotene treated skin.

Measuring STS will determine the location of the enzyme in the epidermis. If the transglutaminase study used liposomes of about 92 kDa\textsuperscript{191}, we expect that the 63kDa molecule of STS will be successful in delivery to the SG/SC via liposomes.

**4.14 LIMITATIONS AND ADVANTAGES OF THE STUDY**

- This study is currently limited to patients with only XLI, who do not form part of contiguous gene syndromes, thus it does not represent all the population with the phenotype of XLI. However, once the treatment reaches clinical trials, it could be evaluated in these patients.

- The study is not controlled with healthy bioengineered skin; therefore future studies should compare the effects of the liposome treatment also on a humanised-normal-skin model.

- The study is limited to the success of the experiments with the rhSTS a) development of an active enzyme, b) which can reach the stratum granulosum, c) which can bind to cholesterol and desulphate it, d) which restores macro and microscopically the morphology of normal skin

- At the moment, the exact effective concentration of STS needed in the liposomes is unknown. Once we know the results of the STS activity, this number can be elucidated.

- The study is also limited to the side effects that liposomes could encounter such as immune recognition\textsuperscript{202}.

- The advantage of this study is that this is the first attempt for delivery of the missing STS in XLI. If the treatment is deemed successful it is expected to significantly increase compliance with treatment and to improve the QoL of patients with XLI.

- Another advantage of this study is that the methodology can be reproduced to attempt the liposomal delivery of other enzymes to the epidermis.

Figure 22 shows a flow chart which summarises this proposed study which aims to provide an easier, simpler understanding of the processes to take place.
Figure 22. Flowchart of proposed study
Chapter 5

“To paint is to pray. To paint is to scream.”
——Oswaldo

DISCUSSION, CONCLUSIONS, RECOMMENDATIONS
Chapter 5:

5.1 DISCUSSION

The differentiation of the epidermis is an important topic for clinicians and scientists working in all fields related to the skin. The concept of how this portion of the integument works is indeed remarkable: a mesh of dead, hard-encased corneocytes are embedded in a tissue which can respond to surface changes and stimulate keratinocyte proliferation and differentiation, provoke immune reactions, increase or decrease gene expression of proteins, lipids, and carbohydrates.¹⁹⁵

Understanding the structure of the skin and its process of cornification has helped in discovering the molecular pathogenesis of many conditions such as the disorders of cornification. And the hope is that with the current understanding of the pathogenesis, relevant therapies will be developed for a specialty that still lacks management options and makes high use of non-specific treatments including emollients, immune-suppressants and retinoids.

Several areas remain to be explored in the common ichthyoses. For instance, an update in epidemiology in the United Kingdom is needed as the latest data is from the 1960's.¹¹⁷ Moreover, all studies regarding ichthyosis epidemiology should be updated considering the new classification from the consensus in Sorèze²⁰ so that results can be divided and reported by ichthyoses type. Also, perhaps even a new classification of these conditions could be done taking into consideration the type of missing molecule (peptide, lipid, carbohydrate) and the most prominent sign of the condition such as epidermal hyperproliferation, scale retention, epidermal barrier abnormality. Another question that arises from the literature review is whether patients with IV have a higher risk of skin cancer because of the FLG mutations and this question must be addressed in future studies.

The ichthyoses are rare and thus the knowledge that dermatologists have about these conditions may be scarce because few consultants will have had contact with the uncommon ichthyoses throughout their career. Also, few studies are published, especially regarding management, for an individual type of ichthyosis. For this reason it is
important to liaise with communities such as the Ichthyosis Support Group (ISG) in the United Kingdom or the Foundation for Ichthyosis and Related Skin Types (FIRST) in the USA. These liaisons have proven important in published papers such as that of Kamalpour et al.\textsuperscript{160} and Styperek et al.\textsuperscript{163} where they have been able to acquire large cohorts of ichthyosis patients through the foundation’s database.

In addition to this, national healthcare systems should encourage the development of focused gatherings so that physicians and patients can learn from each other and together develop relevant trials to finally achieve optimal therapy as well as national guidelines of diagnosis and management according to disease. National guidelines would serve to enhance physician knowledge but, more importantly, they would result in early patient and family education which is imperatively needed. It has been noticed that experienced patients make good use of health resources.\textsuperscript{160,163}

Children with hyperproliferative ichthyoses and other disorders of cornification should be carefully monitored with growth charts. These patients may spend an important amount of energy solely in replacing a defective barrier which could lead to nutritional imbalances and failure to thrive.\textsuperscript{132} A pioneer investigation and the only of its kind, revealed that patients with ichthyoses and failure to thrive have a significant caloric expenditure from the loss of evaporated water with heat from their skin.\textsuperscript{132} As most ichthyoses represent an abnormal barrier with increased TEWL, it is expected that their energy requirements are increased.\textsuperscript{203,204} Indeed a report from 1987,\textsuperscript{205} mentions the improvement in growth and height in patients with severe ichthyoses and successful retinoid therapy.\textsuperscript{184,205} Moreover, the study by Winge et al.\textsuperscript{131} shows that patients with ichthyosis vulgaris can have up to 2292 up-regulated genes, up-regulation that probably requires a significant amount of energy (Figure 15).

Currently the range of therapies for ichthyoses is narrow and generalised though each condition is different from the other.\textsuperscript{188} For instance there are no reviews which summarise the appropriate emollient therapy from one ichthyosis to another. Indeed it would be important to individualise a moisturising formulation for XLI and another one for IV, where one of the conditions requires more keratolysis, and the other needs an aggressive restitution of the skin's barrier.\textsuperscript{188} Correspondingly, compliance with the
treatment is an important consideration because it must be difficult for patients to apply creams three times a day on their skin to only see partial results.

An important limitation to the review of the current therapies for ichthyoses, is that there are scarce trials on any treatment modality for IV. The current understanding of FLG mutations associated to atopic diseases as well as to ichthyosis vulgaris, does pose the question of whether IV could just be a very severe atopic dermatitis. For instance, both conditions are caused by a barrier abnormality and both conditions can show a microscopic image of hypogranulosis because of the lack of FLG. A review noticed that IV can be associated with AD in around 44% of cases and severe IV associates with AD in 76%. Furthermore, patients with IV may be diagnosed and treated as eczema since childhood, treatment that would also have an impact on IV per se. In fact, with the general attention that AD receives, the lack of published trials for IV could be due to the fact that these patients are included in trials of AD which is a more common condition.

While defining new approaches to treatment for XLI, there is an interesting article on a causal treatment for one of the syndromic ichthyosis. Congenital hemidysplasia with ichthyosiform erythroderma and limb defects (CHILD) syndrome has similarities with XLI. For instance, both diseases are linked to the X chromosome, though CHILD is an X-dominant disease. Both diseases show abnormalities of cholesterol metabolism that produce a deficit of cholesterol and an increase of a toxic intermediate, which in CHILD syndrome is C4-methylated and C4-carboxy sterol intermediates, rather than cholesterol sulphate. Paller and colleagues, attempted a novel topical therapy for CHILD where they applied both cholesterol and lovastatin to patients' skin. The purpose of lovastatin, as seen in figure 23, was to reduce the biosynthetic pathway and avoid accumulation of toxic metabolites such as lanosterol. The purpose of the topical cholesterol would be to substitute this lacking lipid, which is an end-product of the metabolic pathway.
The results of the study were stupendous after three months. Although performed only in two individuals, the skin noticeably improved, suggesting this could be a therapy for CHILD syndrome and a similar approach could be used in other ichthyosis including XLI, providing the topical application of an end product along with an upstream pathway inhibitor.\textsuperscript{193}

Unfortunately, in XLI, this approach would not prove effective.\textsuperscript{106} If one attempted to inhibit cholesterol formation altogether, the levels of cholesterol sulphate in the epidermis would be also decreased. As CS is an important inducer of epidermal differentiation markers (Figure 12 in blue), this approach would be imperfect.\textsuperscript{143} Thus, the only solution would be to apply the absent STS to the patients and find a way to reach the SG/SC interface. This would not alter CS in the lower epidermal layers, allowing its important regulatory function.

Thankfully, there seems to be a revamped awareness that the cornification disorders, including the ichthyoses, should be looked at and studied individually.\textsuperscript{143,188} And indeed novel therapies and treatment delivery methods are in development, including a delivery method for filaggrin monomers\textsuperscript{192} that could constitute a therapy for AD and IV, delivery of transglutaminase via liposomes for lamellar ichthyosis,\textsuperscript{191} and the new approach of statins and cholesterol for CHILD syndrome.\textsuperscript{193}

In regards to the proposed study, there are two approaches for the preclinical use of murine models for trials. The first method considers using mice that have been genetically modified to lack an enzyme, in this case a mouse model with a null STS
mutation. However, this model is not available and the cost of creating a null-mouse is significant. Moreover, many times when the null mouse is created, the deletion is fatal in mice, as happened in the case of the transglutaminase-null mouse.\textsuperscript{197} The second option is to graft human skin derived from patients that have the mutation.\textsuperscript{201,207} Attempts to graft full thickness samples have resulted in low graft survival rates,\textsuperscript{207} thus they do not last long enough to be used appropriately to test treatment. So, currently the best option to achieve a good model of a skin condition is to use bioengineering methods\textsuperscript{201} to develop a human skin equivalent from patients and graft this on the back of a mouse.\textsuperscript{208} This humanised skin model, is the best approach to test a medication because of the longer survival rates and the acquisition of the human phenotype in the grafted skin which can be confirmed histologically, ultra-structurally and by immunohistochemistry.

The advantage of the liposomal therapy, proposed in this study, is that this novel delivery method, once perfected, could be applied to a large range of epidermal diseases, and perhaps in other specialty fields as well. For instance, perhaps phosphorylated filaggrin monomers could be delivered via liposomes, with the adjustment to make the molecule ready for uptake by KCs; this would allow the filaggrin function to be substituted in those patients with IV and severe AD who have a FLG-null mutation. Moreover, LEKTI which is a protein that weighs around 42 kDa\textsuperscript{209} could propose an interesting approach to treating Netherton syndrome. LEKTI is a protein that only needs to reach the lower stratum corneum to inhibit the protease activity. Unfortunately, the treatment with liposomes is limited by the size of the molecule. Indeed this approach may not be useful for conditions like Harlequin ichthyosis, where the delivery of the ABCA12 transporter with a molecular weight of 292kDa,\textsuperscript{210} in the same way as profilaggrin, represents a challenge.

Challenges to liposomal therapy do not only include the weight of the molecule; the molecule must be reproduced and tested for activity, the delivery method must be perfected and, as Irvine\textsuperscript{202} comments in his review of a liposomal treatment for filaggrin, it is one thing to do experiments in a laboratory and something completely different applying it to humans. Liposomes could mount an immune reaction in patients that could limit and prevent its use.

Thankfully, the hope is also on genetic therapy, which is currently undergoing great innovation as the present technology is approaching better methods to efficiently
achieve the genetic modification of the cells. In genetic therapy, portions of a gene are delivered by a vector which is usually a virus in the hope that this gene is incorporated into the cell’s own genome. This is a fantastic therapy in writing, however, when it comes to practicality, the treatment poses important risks. If the vector delivers the gene randomly into a cell’s own DNA it could modify an important gene that controls homeostasis in the cell. Therefore, the system is not perfect, because when adding a piece of DNA to incorporate into the human genome, scientists cannot be certain that the vector will only introduce or correct the target gene without altering others.

Indeed for fatal diseases, genetic therapy or any other treatment that improves the condition may be justified, even if one of the treatment’s risks is death. An example of this is the development of a treatment for seven patients with epidermolysis bullosa in which bone marrow transplant was trialed. The therapy deemed successful in the seven children, however two of them died from complication of the transplant. But in studies for these challenging diseases, risks such as death may be overlooked by patients in the hope of finally having a cure for a severely disabling condition.

In diseases which are not fatal or severely disabling, such as XLI, predisposing or increasing the risk for a genomic mutation in any gene important for epidermal homeostasis, is unjustified

In conclusion, the differentiation of the epidermis is a fascinating topic where pioneering advances are being made. Understanding the structure of the epidermis and the process of cornification has brought to light many conditions which were previously termed as of unknown cause. The ichthyoses are a vast range of diseases that yearn for physicians’ attention but do not come to the surface often due to a low incidence. It is hoped that this review has highlighted the importance of the ichthyoses and becomes a foundation for design of future studies and for implementation of guidelines for dermatologists and new resources for patients.
5.2 RECOMMENDATIONS

- Encouragement for the creation and the regular meeting of ichthyoses support groups to develop an easy-access tool for patients and families according to skin condition, which encompasses:
  a) Daily care regarding bath, clothing, leisure activities,
  b) Important information regarding school and activities with peers
  c) Treatments recommended from patients with the same condition and application
  d) Top tips and recommendations
  e) Set of most frequently asked questions
- Creation of focus groups, especially with patients from the most common subsets of ichthyoses to comprehend current therapies and develop relevant, targeted treatments
- Approach to publication with a division of patients response according to disease
- Encouragement for the development of new therapies for all the ichthyoses to increase the range of options that these patients may have
- Encouragement from bursaries and grants for scientists attempting new therapy methods or novel medications
- Participation of multiple centres in trials of medications to further increase validity of results as well as appropriate blinding of patients and evaluators, control with placebo to assess efficacy, inclusion of drop-out patients, and transparent publications of results to decrease or eliminate bias
- Offer of genetic analysis for all patients diagnosed with XLI so that parents are aware of whether or not XLI forms part of a syndrome as well as to define prognosis of the condition


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Marekov LN, Steinert PM. Ceramides are bound to structural proteins of the human foreskin epidermal cornified cell envelope. *Journal of Biological Chemistry* 1998; **273**: 17763-70.


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Dermatologic Therapy

Diaz LZ, Browning JC, Smidt AC

social communication deficits. associated with increased r

Kent L, Emerton J, Bhadravathi V et al. X-linked ichthyosis (steroid sulfatase deficiency) is

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Maya-Nunez G, Cuevas-Covarrubias S, Zenteno JC et al. Contiguous gene syndrome due to
deletion of the first three exons of the Kallmann gene and complete deletion of the

Kent L, Emerton J, Bhadravathi V et al. X-linked ichthyosis (steroid sulfatase deficiency) is
associated with increased risk of attention deficit hyperactivity disorder, autism and social communication deficits. Journal of Medical Genetics 2008; 45: 519-24.


Mazereeuw-Hautier J, Dreyfus I, Barbarot S et al. Factors influencing quality of life in


Sugawara T, Nomura E, Hoshi N. Both N-terminal and C-terminal regions of steroid sulfatase are important for enzyme activity. *Journal of Endocrinology* 2006; 188: 365-74.


Choate K, Dyer JE, Corona RE. *Overview of the inherited ichthyoses*. In: (UpToDate, ed). Waltham, MA. 2014.
Chapter 6:

6.1 APPENDIX 1

Ichthyosis classification, Taken from Choate,\textsuperscript{211} based on Oji \textit{et al.}\textsuperscript{20}

<table>
<thead>
<tr>
<th>INHERITED ICHTHYoses: NON-SYNDROMIC FORMs</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Disease</strong></td>
</tr>
<tr>
<td><strong>COMMON ICHTHYoses</strong>*</td>
</tr>
<tr>
<td>Ichthyosis vulgaris</td>
</tr>
<tr>
<td>Recessive X-linked (nonsyndromic presentation)</td>
</tr>
<tr>
<td><strong>AUTOSOMAL RECESSIVE CONGENITAL ICHTHYosis (ARCI)</strong></td>
</tr>
<tr>
<td>MAJOR TYPES</td>
</tr>
<tr>
<td>Harlequin ichthyosis</td>
</tr>
<tr>
<td>Lamellar ichthyosis\textsuperscript{†}</td>
</tr>
<tr>
<td>Congenital ichthyosiform erythroderma</td>
</tr>
<tr>
<td>MINOR VARIANTS</td>
</tr>
<tr>
<td>Self-healing collodion baby</td>
</tr>
<tr>
<td>Acral self-healing collodion baby</td>
</tr>
<tr>
<td>Bathing suit ichthyosis</td>
</tr>
<tr>
<td><strong>KERATINOPATHIC ICHTHYosis (KPI)</strong></td>
</tr>
<tr>
<td>MAJOR TYPES</td>
</tr>
<tr>
<td>Epidermolytic ichthyosis\textsuperscript{§}</td>
</tr>
<tr>
<td>Superficial epidermolytic ichthyosis</td>
</tr>
<tr>
<td>MINOR VARIANTS</td>
</tr>
<tr>
<td>Annular epidermolytic ichthyosis\textsuperscript{◊}</td>
</tr>
<tr>
<td>Ichthyosis Curth-Macklin</td>
</tr>
<tr>
<td>Autosomal recessive epidermolytic ichthyosis</td>
</tr>
<tr>
<td>Epidermolytic nevi\textsuperscript{§}</td>
</tr>
<tr>
<td><strong>OTHER FORMS</strong></td>
</tr>
<tr>
<td>Loricrin keratoderma</td>
</tr>
<tr>
<td>Erythrokeratoderma variabilis\textsuperscript{¥}</td>
</tr>
<tr>
<td>Peeling skin disease</td>
</tr>
<tr>
<td>Congenital reticular ichthyosiform erythroderma</td>
</tr>
<tr>
<td>Keratosis linearis-ichthyosis congenita-keratoderma</td>
</tr>
</tbody>
</table>

* Often delayed onset (in recessive X-linked ichthyosis mild scaling and erythroderma may be present already at birth).

\textbullet{} Few cases of autosomal dominant lamellar ichthyosis described in literature (locus unknown).

\textDelta{} Also known as \textit{ICHTHYIN} gene.

\textdagger{} \textit{KRT1} mutations are often associated with palmoplantar involvement.

\textsection{} May indicate gonadal mosaicism, which can cause generalised epidermolytic ichthyosis in offspring generation.

\textyen{} Whether progressive symmetric erythrokeratoderma represents distinct mendelian disorders of cornification form is debated.
<table>
<thead>
<tr>
<th>Disease</th>
<th>Mode of inheritance</th>
<th>Gene(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>X-LINKED ICHTHYOSIS SYNDROMES</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Recessive X-linked ichthyosis*</td>
<td>X-linked recessive</td>
<td>STS (and others)*</td>
</tr>
<tr>
<td>Syndromic presentation</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ichthyosis follicularis, atrichia, photophobia (IFAP) syndrome</td>
<td>*</td>
<td>MBTPS2</td>
</tr>
<tr>
<td>Conradt–Hünemann–Happle syndrome (CDPX2)</td>
<td>X-linked dominant</td>
<td>EBP</td>
</tr>
<tr>
<td><strong>AUTOSOMAL ICHTHYOSIS SYNDROMES (WITH)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Prominent hair abnormalities</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Netherton syndrome</td>
<td>Autosomal recessive</td>
<td>SPINK5</td>
</tr>
<tr>
<td>Ichthyosis hypotrichosis syndrome*</td>
<td>*</td>
<td>ST14</td>
</tr>
<tr>
<td>Ichthyosis, hypotrichosis, sclerosing cholangitis syndrome*</td>
<td>*</td>
<td>ERCC2/XPD ERCC3/XPB GTF2H5/TTDA</td>
</tr>
<tr>
<td>Trichothiodystrophy</td>
<td>*</td>
<td>C7orf11/TTDN1</td>
</tr>
<tr>
<td>Trichothiodystrophy (not associated with congenital ichthyosis)*</td>
<td>*</td>
<td></td>
</tr>
<tr>
<td>Prominent neurologic signs</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sjögren–Larsson syndrome</td>
<td>*</td>
<td>ALDH3A2</td>
</tr>
<tr>
<td>Refsum syndrome (HMSN4)*</td>
<td>*</td>
<td>PHYH/PEX7</td>
</tr>
<tr>
<td>Mental retardation, enteropathy, deafness, neuropathy, ichthyosis, keratoderma (MEDNIK) syndrome</td>
<td>*</td>
<td>AP1S1</td>
</tr>
<tr>
<td><strong>Fatal diseases course</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gaucher syndrome type 2</td>
<td>*</td>
<td>GBA</td>
</tr>
<tr>
<td>Multiple sulfatase deficiency</td>
<td>*</td>
<td>SUMF1</td>
</tr>
<tr>
<td>Cerebral dysgenesis, neuropathy, ichthyosis, palmoplantar keratoderma (CEDNIK) syndrome</td>
<td>*</td>
<td>SNAP29</td>
</tr>
<tr>
<td>Arthrogryposis, renal dysfunction, cholestasis (ARC) syndrome</td>
<td>*</td>
<td>VPS33B</td>
</tr>
<tr>
<td><strong>Other associated signs</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Keratitis, ichthyosis, deafness (KID) syndrome</td>
<td>Autosomal dominant</td>
<td>GJB2 (GJB6)</td>
</tr>
<tr>
<td>Neutral lipid storage disease with ichthyosis</td>
<td>Autosomal recessive</td>
<td>ABHD5</td>
</tr>
<tr>
<td>Ichthyosis prematurity syndrome</td>
<td>*</td>
<td>SLC27A4</td>
</tr>
</tbody>
</table>

* Often delayed onset (in RXLI mild scaling and erythroderma may be present already at birth).
• In context of contiguous gene syndrome.
△ Clinical variant: congenital ichthyosis, follicular atrophoderma, hypotrichosis, and hypohidrosis syndrome.
◊ Also known as neonatal ichthyosis sclerosing cholangitis syndrome.
6.2 APPENDIX 2,

Conditions associated with acquired ichthyosis vulgaris, Taken from Okulicz 
et al.\textsuperscript{127}

<table>
<thead>
<tr>
<th>CONDITIONS ASSOCIATED WITH ICHTHYOSIS VULGARIS</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Cancer</strong></td>
</tr>
<tr>
<td>Chronic renal failure</td>
</tr>
<tr>
<td><strong>Hodgkin’s disease</strong></td>
</tr>
<tr>
<td>Nutritional disorders</td>
</tr>
<tr>
<td><strong>Non-Hodgkin’s disease (including mycosis fungoides)</strong></td>
</tr>
<tr>
<td>Human immunodeficiency virus infection</td>
</tr>
<tr>
<td><strong>Kaposi’s sarcoma</strong></td>
</tr>
<tr>
<td><strong>Autoimmune diseases</strong></td>
</tr>
<tr>
<td><strong>Leiomyosarcoma</strong></td>
</tr>
<tr>
<td>Systemic lupus erythematosus</td>
</tr>
<tr>
<td><strong>Carcinoma</strong></td>
</tr>
<tr>
<td>Dermatomyositis</td>
</tr>
<tr>
<td><strong>Breast</strong></td>
</tr>
<tr>
<td><strong>Drugs</strong></td>
</tr>
<tr>
<td><strong>Lung</strong></td>
</tr>
<tr>
<td>Nicotinic acid</td>
</tr>
<tr>
<td><strong>Ovary</strong></td>
</tr>
<tr>
<td>Triparanol</td>
</tr>
<tr>
<td><strong>Cervix</strong></td>
</tr>
<tr>
<td>Butyrophenones</td>
</tr>
<tr>
<td><strong>Leprosy</strong></td>
</tr>
<tr>
<td>Dixyrazine</td>
</tr>
<tr>
<td><strong>Sarcoidosis</strong></td>
</tr>
<tr>
<td>Cimetidine</td>
</tr>
<tr>
<td><strong>Thyroid disease</strong></td>
</tr>
<tr>
<td>Clofazimine</td>
</tr>
<tr>
<td><strong>Hyperparathyroidism</strong></td>
</tr>
</tbody>
</table>
### 6.3 APPENDIX 3,

**Ichthyosis Quality of Life Questionnaire. Taken from Dreyfus et al.**

The following questions concern different periods of time. There are no right or wrong answers. Answer each one as spontaneously as possible by ticking the proposed answer that seems closest to your opinion. If a question does not concern you, tick the corresponding box.

**In the past 4 weeks**

<table>
<thead>
<tr>
<th>Question</th>
<th>Answer Options</th>
</tr>
</thead>
<tbody>
<tr>
<td>1  ... has your skin been red</td>
<td>tremendously a lot a little Not at all not applicable</td>
</tr>
<tr>
<td>2  ... has your skin been sensitive or painful (tense, uncomfortable)</td>
<td>tremendously a lot a little Not at all not applicable</td>
</tr>
<tr>
<td>3  ... have you had dry or thick skin, with a lot of scales (dead skin)</td>
<td>tremendously a lot a little Not at all not applicable</td>
</tr>
<tr>
<td>4  ... has your skin been itchy</td>
<td>tremendously a lot a little Not at all not applicable</td>
</tr>
<tr>
<td>5  ... has your skin hurt because of cracking</td>
<td>tremendously a lot a little Not at all not applicable</td>
</tr>
<tr>
<td>6  ... because of your ichthyosis have your eyes bothered you (dryness, pain, watering, impaired vision, redness)</td>
<td>tremendously a lot a little Not at all not applicable</td>
</tr>
<tr>
<td>7  ... because of your ichthyosis have your ears bothered you (earwax plug, impaired hearing, pain, itching)</td>
<td>tremendously a lot a little Not at all not applicable</td>
</tr>
<tr>
<td>8  ... did your skin have trouble adapting to temperature and/or weather changes</td>
<td>tremendously a lot a little Not at all not applicable</td>
</tr>
<tr>
<td>9  ... have you been bothered by the smell of your skin</td>
<td>tremendously a lot a little Not at all not applicable</td>
</tr>
<tr>
<td>10 ... have you felt like your skin was unsightly because of your disease</td>
<td>tremendously a lot a little Not at all not applicable</td>
</tr>
<tr>
<td>11 ... does the disease make you feel dirty</td>
<td>tremendously a lot a little Not at all not applicable</td>
</tr>
<tr>
<td>... have you felt uncomfortable performing certain everyday actions (such as writing, moving) because of the pain or stiffness caused by ichthyosis</td>
<td>tremendously a lot a little Not at all not applicable</td>
</tr>
<tr>
<td>12 ... have you felt “fatigue” in connection with your ichthyosis</td>
<td>tremendously a lot a little Not at all not applicable</td>
</tr>
<tr>
<td>13 ... has your scalp bothered you (combing, hair care, pain, or itching)</td>
<td>tremendously a lot a little Not at all not applicable</td>
</tr>
<tr>
<td>14 ... have you performed all desired activities (sports and leisure without fear that others might see your skin)</td>
<td>tremendously a lot a little Not at all not applicable</td>
</tr>
<tr>
<td>15 ... have you changed your vacation plans or the places you go because the planning required by your ichthyosis was too complicated</td>
<td>tremendously a lot a little Not at all not applicable</td>
</tr>
<tr>
<td>16 ... have you felt that your ichthyosis was a handicap (aesthetic or physical) even if you do not consider yourself a handicapped person</td>
<td>tremendously a lot a little Not at all not applicable</td>
</tr>
<tr>
<td>17 ... have you experienced mood swings because of ichthyosis</td>
<td>tremendously a lot a little Not at all not applicable</td>
</tr>
<tr>
<td>18 ... have you felt sad, discouraged or powerless in the face of your disease</td>
<td>tremendously a lot a little Not at all not applicable</td>
</tr>
<tr>
<td>19 ... have you felt lonely or withdrawn because of the disease</td>
<td>tremendously a lot a little Not at all not applicable</td>
</tr>
<tr>
<td></td>
<td></td>
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<td>... have you experienced a feeling of anger, being fed up, a sense of injustice because of your disease</td>
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<td>... have you felt afraid of the future (treatment losing their effectiveness, worsening, difficulty applying the creams with age)</td>
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<td>... have you felt afraid that the disease could restrain a romantic/sexual relationship</td>
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6.4 APPENDIX 4

Patient Information Leaflet

Study title

The Use of Steroid Sulphatase Delivered in Liposomes as a Novel Therapy for X-Linked Ichthyosis

Invitation to participate in the study

We would like to invite you to take part in an experimental study that is investigating a treatment for X-linked ichthyosis. Before you decide to participate, it is essential that you understand why this research is being done and what it would involve. Please read the following information carefully, and discuss it with your family, friends or GP if you wish to do so. Feel free to contact us if there is something that is not clear or if you have further queries.

What is the purpose of this study?

X-linked ichthyosis is a common skin condition, and it affects many patients just like you. Unfortunately, so far there are no effective treatments for X-linked ichthyosis, and patients many times feel exhausted from utilising creams of all sorts that improve the skin very minimally. Furthermore, as you may have experienced, patients with ichthyosis have to apply creams more than twice per day which can become tiring and can take a significant amount of the day. In X-linked ichthyosis, patients are missing an important protein called steroid sulphatase; the absence of this protein is what makes the skin look thick, dark and scaly. Thus far, no one has tried to apply the lacking protein to the skin of patients as a treatment.

The purpose of this study is to develop a good way to apply the steroid sulphatase onto the skin of patients with X-linked ichthyosis. This study will use something called liposomes, which are small balls that will contain the protein in its interior. However, as this is a very new treatment option we cannot apply it on humans yet. For this reason, we are looking for patients with X-linked ichthyosis to donate a very small piece of their skin (5mm) so that we can try the treatment on a lab, on mice.
The liposomes have already been used in a similar experiment for patients with lamellar ichthyosis, and it has shown success. Therefore, this study wants to find out whether the treatment will also work for the skin condition that many patients, like yourself, have.

**Why have I been invited for this study?**
You have been invited as a possible candidate for this study because you have been diagnosed with X-linked ichthyosis.

**Do I have to participate in this study?**
It is entirely up to you to decide whether or not to take part in this study. If you do decide to take part, you will be asked to sign a consent form that confirms your voluntary participation in this study. You will be provided with a copy of the signed consent form and a copy of the information sheet to keep. You are free to withdraw at any time and are not obliged to give a reason for doing so. The standard of care you receive will be unaffected by your decision to not participate or withdraw from the study.

**What will happen to me if I take part of the study?**
If you decide to take part, you will undergo initial evaluation which includes a detailed medical history and physical examination. You will also have blood withdrawn once, to perform tests to determine that you meet all the required eligibility criteria. The tests includes a genetic analysis of a portion of your X-chromosome, as well as an analysis of two other mutations that are related to ichthyosis. We will also check how active your protein, steroid sulphatase is. You may have had this last exam done before, but we need to double check for the purpose of the study.

If you meet the eligibility criteria you will undergo a skin biopsy. This will be a small punch of 5mm taken from your right or left buttock under anaesthesia. You should not apply any topical preparations four weeks before having the biopsy

**What do I have to do?**
You must provide the doctor with information on all the medications you are applying to your skin or taking orally. You must agree not to use any topical medication four weeks before having the biopsy. Once we have your skin sample, you will not need to do anything else. All other experiments will be carried out in the laboratory. Keep the dressing on the wound for 24 hours and then apply a plaster if it is still bleeding.
What will happen to my skin?
Your skin will be used to grow a patch of human skin on the back of mice, and it will be used to test whether it is possible to put the steroid sulphatase protein back into skin using the liposomes, which will be applied every other day for 14 days. An evaluator will check if the skin improves with the therapy. The mice are treated with approval from the Animal Care and Ethics Committee; and it abides to British laws and regulations of experimental procedures.

What are the side effects of the procedure?
You will have a small scar from the site of the skin biopsy. All side effects which can be expected will be given to you in the consent form, if you do meet the criteria for the study.

How long will I need to be available for the study?
On your part, you will have to visit the dermatology department twice. The first visit would be to perform a clinical consultation and to draw blood. The second visit would be to perform the biopsy, if you so agree.

What are the possible benefits from taking part in the study?
We are currently looking for a good treatment for X-linked ichthyosis, but this treatment is not yet ready to be tested in humans. Therefore, there is no immediate benefit for you on the trial. However, the information obtained from this study will prove useful for the development of new treatments for X-linked ichthyosis.

What are the possible risks and disadvantages of taking part?
The possible risk is to have a small scar from the site where we take the skin biopsy.

What if there is a problem?
In the extremely unlikely event of any irreversible side effects resulting from taking the biopsy, please be advised that there will be no special compensation awarded to you. Any complaint about the way you have been dealt with during the study or any possible harm to you will be addressed via the normal NHS complaints mechanism. After we take the biopsy, we do not expect any problems, and we will only use the skin for this experiment which you are consenting to.

Will my taking part in the study be kept confidential?
Your medical information will only be accessed by authorised personnel and used only for research purposes.
What will happen if I don’t want to carry on with the study?
You are free to withdraw at any time, and are not obliged to give a reason for doing so. The standard of care you receive will be unaffected by your decision to withdraw from the study.

What will happen to the results of the research study?
All data will be collected and analysed. The data can be made available to you upon request from the research team. The information may also be used for future research, medical publications, presentations and education. You will not be identified in any such presentation or publication, unless you have consented to do so.

Has anyone reviewed the safety and ethical issues concerning this study?
This study is reviewed by a group called the Research Ethics Committee. The experiment on mice have been approved by the Animal Care and Ethics Committee.

For further queries please contact the study doctor below.

Dr. ..................
Department of Dermatology
Tel. ...........

Thank you for reading this information sheet and take your time to consider whether or not you would like to enter the study.
6.5 APPENDIX 5

Consent Form

Centre number
Study number
Patient identification number for this trial

Study title
The Use of Steroid Sulphatase Delivered in Liposomes as a Novel Therapy for X-Linked Ichthyosis

Principal investigator  ..................

- I consent that I have read and understood the information sheet dated...............for the above study. I have had the opportunity to consider the information, ask questions and have had these answered satisfactorily

- I understand that my participation is voluntary and that I am free to withdraw at any time without giving any reason, without my medical care or legal rights being affected

- I agree to providing a medical history and undergoing physical examinations performed by the medical staff conducting the trial.

- I agree to have a blood test performed, and I understand the tests that will be performed on my blood.
• I agree to have one 5mm punch biopsy taken from my skin, from the right or left buttock. I understand that the biopsy will be taken by the study staff after injecting local anaesthesia.

• I understand that relevant sections of my medical notes and data collected during the study may be looked at by individuals from the regulatory bodies or from the NHS trust, where it is relevant to my taking part in this research. I give permission for these individuals to have access to my records.

• I agree to my GP being informed of my participation in the study.

• I declare that I am not taking part in any trial or study at this time.

• I agree to take part in the above study.

_________________                           __________   _______________
Name of the patient                                 Date                  Signature

_________________                        __________________
Name of the investigator                        Date                 Signature

taking consent