Analysis of the structure and function of the epidermal barrier in patients with ichthyoses—clinical and electron microscopical investigations

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Abstract

Background Ichthyoses are pathogenetically characterized by a pronounced disorder of the epidermal barrier. Clinically, hyperkeratosis, severe scaling and erythroderma are present on the entire integument. The time-consuming therapy includes daily baths and the application of skin care products to restore the epidermal barrier.

Objectives and methods To enhance the knowledge about the structure and function of the epidermal barrier in ichthyoses, we conducted clinical, biophysical and electron microscopical measurements on 46 patients with ichthyoses, including autosomal recessive ichthyoses, keratinopathic ichthyoses, X-chromosomal-recessive ichthyosis and Netherton syndrome.

Results The patients displayed a significantly decreased skin hydration along with unexpectedly low transepidermal waterloss values. Electron microscopical examinations demonstrated a severe occlusion of the epidermis by lipid remnants of skin care products in the stratum corneum. We found decreased intercellular lipid lamellae and an increased undulation of the corneocyte membrane of all ichthyoses, mostly pronounced in Netherton syndrome. The lipid profiles of ichthyoses showed decreased esterified $\omega$-hydroxy-sphingosine (EOS) ceramide levels.

Conclusions The results demonstrate the extent of the epidermal barrier disruption in ichthyoses. In combination with the knowledge about pathogenetic causes, individually improved therapeutic options can be derived from our results. In the future, the analyses of the organization of intercellular lipid lamellae and corneocyte membrane undulation will enable improved investigations of the epidermal barrier in ichthyoses and may be used to study and evaluate possible effects of topical skin preparations.

Received: 12 September 2021; Accepted: 15 December 2021

Conflicts of interest

The authors declare that they have no conflicts of interest regarding this work.

Funding sources

Main support: Project funding by European Academy of Dermatology and Venereology (EADV), (n° PPRC-2019-22).
Further support: German Foundation of Paediatric Dermatology.

Introduction

Ichthyoses are hereditary cornification disorders of the skin, characterized by a disruption of the epidermal barrier.1 The total prevalence of rare congenital ichthyoses in Germany is 10 : 1 million.2 Non-syndromic forms, that are limited to the skin, are distinguished from syndromic ichthyoses with systemic manifestations,3 such as Netherton syndrome (NTS).

The epidermal barrier is localized in the stratum corneum (SC).4,5 Corneocytes form a resistant cell bond, with hydrophobic lipids filling the intercellular space (ICS).6,7 organized in intercellular lipid lamellae (ICLL). Various mutations of genes encoding for proteins of the epidermal barrier are known to cause ichthyoses.8 NTS is caused by mutations of the protein LEKTI that regulates proteases of the desquamation of corneocytes.7,9 Keratinopathic ichthyoses (KPI) result from mutations in keratin genes of the cytoskeleton that leads to a loss of stability and integrity of the SC.7,10 Autosomal recessive ichthyoses (ARCI) include all recessively inherited ichthyoses with other genetic causes.5,11 The most common cause are mutations of transglutaminase 1 (TGM1),12 the key enzyme in the assembly of the
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Materials and methods

Study subjects and study design
The study was approved by the local ethics committee of the medical faculty, Christian-Albrecht University of Kiel, Germany, on 22 February 2019 (D431/19). Written and informed consent was obtained from all study members or their parents in case of minors before the beginning of the investigations. The investigations of patients with ichthyoses were conducted at the annual meeting of the German self-help group of ichthyosis on 22/23 March 2019 in Mücke-Flensungen, Germany. The control group was tested at the Department of Dermatology, University of Kiel, Germany, on 29 June 2019. In total, 56 adults and children, aged 1–56 years, were included (40 female, 16 male): 10 skin-healthy controls, 11 patients with KPI, 27 patients with ARCI, 7 patients with NTS and 1 patient with XRI. The data of 6 patients with NTS were included from previous investigations. The examinations were performed on the volar arm side-by-side on lesional (L) and non-lesional (NL) skin, whenever possible to distinguish.

Anamnesis and clinical severity
Anamnestic features included age, sex, type of ichthyosis and genetic information, age of onset, birth complications, family history, previous condition and therapy. Due to the lack of a universal tool for assessing the clinical severity of ichthyoses, we used the SCORAD index (severity scoring of atopic dermatitis) in combination with visual analog scales (VAS) for assessing disease severity and the level of itch intensity and sleep-loss. An adapted SCORAD index was calculated by using the formula of the SCORAD, counting in only the following intensity items that match ichthyotic skin: "erythema", "dryness" and "oozing/crusts". These three items were also analyzed separately (rating from 0 = "absent" to 3 = "severe").

Biophysical measurements
To indirectly evaluate the properties of the epidermal barrier, biophysical measurements by means of SC hydration and TEWL were performed, using Corneometer® CM 825 and Tewameter® TM 300 (Courage & Khazaka). Due to the outpatient setting at the venue of the investigations, standardized methods as described by Courage & Khazaka could not be consistently achieved. Mean (+ SD) air temperature and humidity were 24.4°C (±1.7°C) and 50.4% (±8.8%) at the testing site in Mücke and 28.8°C (±1.3°C) and 51.7% (±7%) while testing the control group.

Intercellular lipid lamellae organization and corneocyte membrane undulation
For direct investigations of the epidermal barrier, TEM was used. Harvesting SC samples by the non-invasive Lipbarvis® method and subsequent processing for TEM analysis was...
performed as described in the previous studies by Dähnhardt-Pfeiffer et al.\textsuperscript{24,30} The length of the ICL (nm) was measured in a defined space of the ICS (nm\textsuperscript{2}) and the ratio of ICL/ICS normalized to 1000 nm\textsuperscript{2}, which results in the normalized intercellular lipid lamellae (nICLL).\textsuperscript{30} To capture corneocyte membrane undulation, membrane length (µm) and distance (µm) between two marks on the corneocyte membrane was measured morphometrically during TEM analysis (Fig. 1). Measurements were performed repetitively, and the ratio of undulation (RoU) was calculated as the quotient from distance to length. The RoU is an additional marker to quantify epidermal barrier disruption.\textsuperscript{31}

**Intercellular lipid composition and lipid profile analyses**

The intercellular lipid composition of the SC was quantified by TEM analysis in combination with high-performance thin-layer chromatography (HPTLC) as described by Dähnhardt-Pfeiffer et al.\textsuperscript{24} It included the lipid cholesterol (CHOL), free fatty acids

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**Figure 1** Measurement of the ratio of undulation (RoU). Transmission electron microscopic (TEM) pictures by Microscopy Services\textsuperscript{®}. Corneocytes of healthy skin have a flat, oval shape (a). In contrast to this, corneocytes of ichthyotic skin show an undulated corneocyte membrane (b). Membrane length (µm) and distance (µm) between two marks A and B on the corneocyte membrane was measured morphometrically on healthy skin (c) and on ichthyotic skin (d). Measurements were performed repetitively, and the ratio of undulation (RoU) was calculated as the quotient from distance to length.
(FFA) as well as the ceramides (Cer) esterified \(\Omega\)-hydroxysphingosine ceramide (EOS), non-hydroxy pyrophosphingosine ceramide (NP) and non-hydroxy 6-hydroxysphingosine ceramide (NH).\(^\text{15}\) Additionally, an individual lipid profile analysis was performed for each subject by densitometrically assessing the raw data of the HPTLC. This enabled the analysis of lipid ratios, especially the ratios of CHOL to FFA to Cer EOS.

**Statistical analysis**

The collected data were analyzed using IBM SPSS Statistics (version 26.0 and 27.0) and RStudio software (version 1.4.1106). For all investigations, the included number (n) of participants was given, and groups with a size \(\geq 5\) were included in the statistical analysis. After checking Q-Q diagrams, the assumption of normal distribution had to be declined for all data. Descriptive statistics for nominal data were given by absolute and relative frequencies, for continuous data by median with first and third quartile. Associations between two groups were assessed by means of the \(\chi^2\) test or Fisher’s exact test, and for more groups by the Kruskal–Wallis test, which was then adjusted for multiple testing by Bonferroni correction. All tests were performed two-tailed, and the significance level was set at \(P < 0.05\) in an explorative manner.

**Results**

Tables with all continuous and nominal values of the results can be found in the Tables A1 and A2.

**Anamnestic features**

There was a significant difference between the age of the ichthyosis group and the control group (\(P = 0.004\)), and the distribution of sex showed no difference (\(P = 1\)). Among the ichthyoses, age distribution showed no difference (\(P = 0.653\)), neither did the distribution of sex (\(P = 0.051\)). The genetic causes of the included ichthyoses were very heterogenous. In the ARCI group, mutations of TGM1 were most frequent (10 of 27, 37%), followed by mutations of CYP2F44 (3 of 27, 11%), and 33% (9 of 27) were not yet genetically identified. In the KPI group, mutations of KRT10 were most common (8 of 11, 73%). In total, 15% (6 of 40) of the ichthyosis group was born as a colloidon baby, most of whom with ARCI. Concerning the distribution of pre-existing conditions, the control group showed a significantly higher number of allergic rhinitis (\(P = 0.023\)) and the history of atopy in childhood (\(P = 0.006\)). In the ichthyosis group allergies were most common (8 of 40, 20%). Treatment was similar in all groups of ichthyoses, and no significant differences could be detected. 90% (36 of 40) of the participants used balneotherapy weekly to daily, and 92.5% of all subjects with ichthyosis (37 of 40) applied daily skin care products. Emollients on a lipophilic base with different moisturizing additives such as urea (80%, 32 of 40) were mostly used.

**Clinical severity**

The ichthyosis group in total showed an elevated severity level for SCORAD but no differences could be detected among the ichthyoses KPI and ARCI, neither by SCORAD (\(P = 0.11\)), nor by adapted SCORAD (\(P = 0.987\); Fig. 2a). The separate evaluation of the intensity categories showed no significant differences except for the category “oozing/crusts”, where higher values for the KPI compared with ARCI (\(P = 0.003\)) could be detected (Figs 2b–d). Itch intensity and sleep-loss showed no differences between the ichthyoses KPI and ARCI (Fig. 3).

**Biophysical measurements**

The results of TEWL of the ichthyosis group showed no significant differences compared with the control group (\(P = 0.457\) L, \(P = 0.977\) NL). Nevertheless, the results of TEWL showed a broad variation, especially in the ARCI group. The skin hydration was significantly lower in the ichthyosis group, both on L and NL skin (\(P < 0.001, P < 0.001\)). When comparing KPI with ARCI, neither TEWL (\(P = 0.506\) L, \(P = 0.063\) NL) nor hydration (\(P = 0.663\) L, \(P = 0.751\) NL) showed any differences, but again ARCI showed a wider range of hydration (Fig. 4).
Results of biophysical measurements showed significant differences for skin hydration between ichthyoses to controls \( (P = 0.668) \) and sleep-loss \( (P = 0.439) \) showed no significant differences. KPI, keratinopathic ichthyoses; ARCI, autosomal recessive congenital ichthyoses; NTS, Netherton syndrome; XRI, X-chromosomal-recessive ichthyoses; n, participants. \(* P > 0.05; ** P < 0.05; *** P < 0.001.\)

**Corneocyte membrane undulation**

The RoU of the ichthyosis group showed significant differences compared with the control group \( (P < 0.001) \) and among KPI, ARCI and NTS \( (P < 0.001) \). The RoU of NTS was significantly higher than the RoU of ARCI \( (P < 0.001) \), and the RoU of KPI was marginally higher than the RoU of ARCI \( (P = 0.05) \). There was no difference between the RoU of KPI and NTS \( (P = 0.58) \); Fig. 7.

**Intercellular lipid composition and lipid profile analyses**

Due to the earlier described difficulties during preparation for TEM analysis, chromatographic analysis could be performed on 15 samples in the ichthyosis group. Owing to the lipid remnants, robust results could be obtained only for CHOL, FFA and ceramides, and a differentiation of the fatty acids was not possible. Statistical comparisons of the lipids were not possible; therefore, individual lipid profiles were densitometrically interpreted. The individual lipid profile analyses of 15 subjects with ichthyoses clearly showed similarities. In 73% (11 of 15) of the samples, the ratio of Cer EOS to CHOL was altered, showing decreased amounts of Cer EOS. Additionally, 47% of lipid profiles (7 of 15) showed increased amounts of FFA. In three profiles, a double peak of the CHOL could be detected and two more profiles showed a shoulder of the CHOL peak (Fig. 8).

**Discussion**

**Corneocyte membrane undulation**

Morphological parameters of corneocytes, although long studied, have not yet been established as routine parameters to describe the epidermal barrier.\(^{32}\) An undulated corneocyte membrane was mentioned as an additional finding by Fartasch et al.,\(^ {33} \) but not investigated in detail. Our significant findings of the RoU between the ichthyosis group and the controls, as well as among the ichthyoses, now suggest that the RoU is a suitable parameter to describe the epidermal barrier disruption in ichthyoses. Previous measurements on different skin types showed an undulation of 1.09 in healthy skin, slightly increased RoU of 1.13 in atopic dermatitis and a significantly higher RoU of 1.67 in ichthyosis vulgaris.\(^ {34} \) NTS showed the highest undulation in our study, with a significant difference to the undulation of ARCI. This resembles the strong epidermal barrier damage of sunken deeply into the clefts and formed thick, lamellar lipid plaques (Fig. 5). Due to the product lipid remnants, preparation for TEM analysis could only be conducted on 32 samples in the ichthyosis group. Compared to the control group, all ichthyoses showed a significant reduction of nICLL both on L and NL skin \( (P < 0.001) \). Among the ichthyoses, no significant differences could be detected \( (P = 0.11 L, P = 0.068 NL) \). The nICLL of NTS were lowest, followed by KPI and ARCI. Generally, ARCI demonstrated the broadest range of results (Fig. 6).

**Intercellular lipid lamellae organization**

Morphological TEM analysis showed large clefts in the SC of all patients with ichthyosis, and remnants of skin care products had
NTS, also reflected by the low nICLL and often described in literature.7,19 The pathogenetic reason for the variable expression of the corneocyte membrane undulation in barrier defects has not yet been investigated. Epidermal barrier disruption is caused by structural defects either of the lipids or the corneocytes in the SC.34 Possibly, corneocyte membrane undulation could be connected to ultrastructural changes of the cytoskeleton, especially since the ichthyoses KPI and NTS showed particularly highly...

Figure 5  Product lipid remnants in stratum corneum (SC) of ichthyoses. Transmission electron microscopic (TEM) pictures by Microscopy Services©. Lipid remnants (yellow circles) from skin care products complicated the processing of SC samples for TEM and high-performance thin-layer chromatography (HPTLC) analysis (a). Remnants of topically applied lipids had sunken deeply into clefts of the SC and formed thick lamellar lipid plaques (yellow lines) that showed broader distances than the physiological lipid lamellae of the SC (b).

Figure 6  Normalized intercellular lipid lamellae (nICLL) in comparison with ichthyoses to controls (a) and among ichthyoses (b). Ichthyoses showed significantly reduced nICLL on lesional and non-lesional skin compared with controls (P < 0.001). Among KPI, ARCI and NTS, nICLL differed marginally significantly (P = 0.068 NL, P = 0.11 L). KPI, keratinopathic ichthyoses; ARCI, autosomal recessive congenital ichthyoses; NTS, Netherton syndrome; XRI, X-chromosomal-recessive ichthyoses; n, participants. *P > 0.05; **P < 0.05; ***P < 0.001.

Figure 7  Ratio of undulation (RoU) among ichthyoses. In comparison with healthy skin (RoU = 1.09), the RoU of ichthyoses was significantly elevated (P < 0.001). Among KPI, ARCI and NTS, the RoU showed significant differences (P < 0.001). KPI, keratinopathic ichthyoses; ARCI, autosomal recessive congenital ichthyoses; NTS, Netherton syndrome; XRI, X-chromosomal-recessive ichthyoses; n, participants. *P > 0.05; **P < 0.05; ***P < 0.001.
Figure 8  Individual lipid profiles. HPTLC images by Microscopy Services©. The individual lipid profiles were densitometrically assessed from the raw data of the HPTLC. Lipid profile A (ARCI) shows lower amount of Cer EOS to CHOL and a higher amount of FFA. Lipid profile B (KPI) has an increased amount of CHOL with a normal ratio of Cer. The lipid profile C (ARCI) shows a shoulder in the CHOL peak (arrow), whereas lipid profile D (ARCI) shows a double peak of CHOL and elevated levels of FFA. KPI, keratinopathic ichthyoses; ARCI, autosomal recessive congenital ichthyoses; HPTLC, high-performance thin-layer chromatography; CHOL, cholesterol; FFA, free fatty acids; Cer, ceramides; EOS, esterified ω-hydroxy-sphingosine; NL, non-lesional; L, lesional.
undulated corneocytes. In NTS, the deficiency of LEKT1 leads to premature degradation of the corneodesmosomes with subsequently increased desquamation. KPI are caused by mutations of keratins that contribute to the mechanical stability of corneocytes. The pathogenetic causes of ARCI and XRI, on the contrary, are predominantly involved in lipid synthesis. Further on, the marginally significant difference of the RoU between ARCI and KPI could be due to the rejection of the normal distribution of the data.

**Intercellular lipid lamellae organization**

The organization of intercellular lipids as lipid lamellae in the SC is a main component of an intact epidermal barrier and altered in ichthyoses. In our study, the significant decrease of nICLL of the ichthyosis group compared with the controls resembles the extent of barrier disruption in ichthyoses. Significantly decreased numbers of ICLL have been described for ichthyoses. Compared to atopic dermatitis (nICLL 40 nm/1000 nm2), the median of the ichthyosis group in our study is slightly higher, which could be due to the large variability of the results. In our study, lowest nICLL were found for NTS, for which qualitatively and quantitatively reduced ICLL are known as an expression of the severe epidermal barrier disruption. The reduced nICLL of KPI could match with previously described reduced and fragmented ICLL. For ARCI, ultrastructural differences between the subtypes in the architecture of the ICLL have been described, which may correspond to the large variability of the nICLL of ARCI in our study. Thus, the most frequent cause of ARCI-LI are mutations of TGM1, which result in disrupted linking of the ICLL to the CE, and fragmented and shortened ICLL. In contrast, ARCI-CIE show a large variability in electron microscopical investigations, with the formation of a locally excessive, disorganized or absent lamellar structure and reduced secretion of lamellar bodies. Genetically, various mutations involved in the lipid synthesis or transport of lamellar bodies are known. The marginally significant differences of nICLL among the ichthyoses ARCI, KPI and NTS in our study could result from the rejection of the normal distribution of the data.

**Intercellular lipid composition and lipid profile analyses**

The ratio of intercellular lipids is essential for the formation of the ICLL and thus the integrity of the permeability barrier in the SC. Among lipids, the presence of ceramide EOS in particular contributes decisively to the formation of the ICLL. In our study, we found reduced Cer EOS levels in most of the lipid profiles with ARCI. In literature, reduced Cer EOS levels, especially Cer EOS and NP, are also described for ARCI-LI. Pathogenetically, various mutations of ARCI are involved in lipid pathways: CYP4F22 enzyme or CERS3 are engaged in the synthesis of ultralong-chain ceramides, and the transport protein ABCA12 regulates the storage of ceramides in lamellar bodies. In our study, two individuals with reduced Cer EOS levels displayed mutations of the CYP4F22 enzyme; therefore, the hydroxylation of ultralong-chain ceramides in the SC is interrupted and Cer EOS cannot be synthesized. These patients with mutations in the lipid pathway could particularly benefit from targeted applications of deficient lipids to restore a balanced lipid ratio. In contrast to synthetic lipids, physiological lipids such as ceramides can be taken up by keratinocytes and integrated in the formation of the ICLL.

For other subjects of our study that showed reduced Cer EOS in the lipid profile, either mutations of TGM1 were present or genetic mutations had not yet been identified. TGM1 is the key enzyme in the assembly of the CE, but it is controversially discussed whether it is also involved in the linkage of ICLL to the corneocytes. The excessive amount of FFA in the lipid profiles could be due to remnants of skin care products stored in the SC. Although an increased synthesis of FFA is part of stereotypic repair mechanisms that are activated in ichthyosis, rather low FFA levels are described for ichthyoses such as LI and NTS. The shoulder of CHOL in the lipid profile of two individuals could also be interpreted as FFA from product remnants. The double peak of CHOL in three lipid profiles could correspond to product remnants or an increased proportion of cholesterol sulfate. The increased presence of the cholesterol precursor could also be an indicator for permanently activated repair mechanisms by means of an increased cholesterol synthesis. In XRI, however, the increase in cholesterol sulfate results from the deficiency of STS. Our findings should be verified and deepened in further studies.

**Biophysical measurements**

The measurement of TEWL and skin hydration is generally well suited to quantify epidermal barrier damage in ichthyoses, with TEWL values correlating with the extent of epidermal barrier disruption. Previous studies mainly described significantly increased TEWL values together with significantly decreased skin hydration for ichthyoses compared with healthy skin. In contrast, in a study conducted on 16 Korean patients with XRI, Lee et al. found normal TEWL values along with reduced skin hydration. Their findings suggest a compensation of the epidermal barrier disruption due to a gene polymorphism of serine proteases. This may be an ethnical specificity of the Eastern compared with the Western population. Similar to the aforementioned study, we did not observe a difference regarding TEWL, although skin hydration of the ichthyosis group was significantly decreased compared with the controls. A possible explanation for the low TEWL values could be the continuous application of topical preparations (90% of the patients used topical preparations on a daily basis). However, galenic bases develop an intrinsic effect after application onto the skin, due to segregation and evaporation of volatile components such as water. The remaining lipophilic components lead to occlusion and, with frequent application, to a
negative water balance of the skin.\textsuperscript{47} Therefore, the excessive application of externa by patients led to the occlusion of the skin and consequently altered TEWL values.\textsuperscript{48,49} Furthermore, the low skin hydration suggests only a short-term effect of hygroscopic additives on the SC of ichthyoses. Given a lack of data due to small sizes of study cohorts, only few comparisons of biophysical measurements among ichthyoses exist to this date.\textsuperscript{43}

Significant differences of TEWL values were only described comparing NTS or ARCI-GIE subtype to epidermolytic ichthyoses, but not to ARCI-LI subtype.\textsuperscript{45,50} The lack of difference in our study could therefore result from the clinical heterogeneity of ARCI, which is also reflected by the large variability of ARCI results.

Interestingly, the occlusion of the epidermis observed in the biophysical measurements could correlate with the lipid remnants from skin care products in the SC of all ichthyoses that were seen during TEM analysis. One could have defined the application of externa prior to the investigations as an exclusion criterion, but since their daily application (for infants up to eight times per day) are guideline recommendations,\textsuperscript{1} their restriction over several days would ethically not be justifiable. Thus, the limitations of our study highlight the urgent need to optimize therapy for ichthyoses. To improve the biophysical measurements and the evaluability of SC samples in TEM analysis, it is recommendable to determine the examination site on the skin beforehand to exclude this area from skin care product application prior to testing. Another limitation of the biophysical measurements lay in the out-of-hospital setting without the possibility to standardize environmental conditions. Biophysical measurements can be influenced by environmental confounders.\textsuperscript{29,51,52} Especially, high skin hydration of the controls could be biased by perspiration.\textsuperscript{49} Furthermore, positive history of childhood atopy in the control group could be an explanation for the outlier of TEWL.\textsuperscript{53} To verify the results of our study, measurements should be repeated under standardized conditions. Additionally, in future out-of-hospital settings, a modern instrument that is insensitive to environmental conditions and easier to handle could be used instead of the conventional Tewameter.\textsuperscript{48,54}

Clinical severity

In terms of clinical severity, the ichthyoses ARCI and KPI showed no significant differences, except for the criteria of "oozing/crusts". Our evaluation is consistent with the use of other scores that also identified differences between ichthyoses only when evaluating individual criteria,\textsuperscript{45,50} especially with oozing blisters being typical for KPI.\textsuperscript{7} As far as known, there is no consensus on assessing the clinical severity of ichthyoses.\textsuperscript{25} While previously developed scores are limited to certain phenotypes,\textsuperscript{50} a suitable score should reflect the typical morphology and phenotypic variability of all ichthyoses and be able to differentiate between ichthyoses. In light of this, Bodemer et al.\textsuperscript{55} modified the SCORAD index by adding ichthyosis-specific criteria and taking into account the influence on the quality of life.\textsuperscript{55} Therefore, we used the established SCORAD index\textsuperscript{55} with an adaptation for ichthyoses and also evaluated itch and sleep-loss as criteria of subjective limitations. Itch is an associated symptom of ichthyoses with a negative impact on quality of life and sleep.\textsuperscript{56–58} The absence of differences between the ichthyoses KPI and ARCI regarding itching and sleep-loss is consistent with literature\textsuperscript{56}; however, the results were in a lower range than expected.\textsuperscript{55,59} A likely reason is that parents of infants evaluated their subjective impact. Several studies on itch of children with atopic dermatitis found that the parents’ objective assessment of itch differed from the children’s subjective assessment.\textsuperscript{60} For the same reason, the evaluation of sleep-loss of infants in our study may also be inaccurate. Until a general clinical score for ichthyoses is found, the evaluation of the clinical severity can be complemented by other methods such as biophysical measurements to obtain an overall picture.\textsuperscript{25}

Study group and anamnestic features

The relatively small number of included patients with ichthyoses could be a limiting factor of the study. However, taking into account the rareness of congenital ichthyoses,\textsuperscript{1,2} the study group is of meaningful size. Furthermore, the study group resembles the well-known genetic heterogeneity of the included ichthyoses, particularly of ARCI.\textsuperscript{7,11} Consistent with literature is also the proportion of patients with ichthyoses of which the genetic cause has not yet been identified.\textsuperscript{1,2,3,4,61} This fact highlights the still existing knowledge gap concerning the pathology of ichthyoses. Among ARCI, the frequent appearance of a collodion baby at birth is typical\textsuperscript{61,62} and illustrates the clinical severity of the included patients. Concerning medical history, allergies were most frequent in the ichthyosis group. Due to the disruption of the epidermal barrier, allergens can penetrate the skin more easily, which may cause atopic tendencies or allergies.\textsuperscript{18} For NTS in particular, an atopic predisposition is known.\textsuperscript{12}

Due to difficulties to recruit children for the control group, a significant age difference between the ichthyosis group and the control group could be observed. However, the low median age of the control group illustrates the adolescent age of the controls. Additionally, previous biophysical\textsuperscript{23,48} and electron microscopical investigations\textsuperscript{30} showed no differences between children and adults.

In summary, the epidermal barrier of ichthyoses can be well characterized by electron microscopic investigations. Taken together, the analyses of nICLL and RoU depict both components of the SC, intercellular lipids and corneocyte morphology. In combination with the individual lipid profile analyses and the knowledge of the pathogenetic causes of ichthyoses, new implications for topical therapy can be derived. In the future, these investigations can be used to evaluate the effects of topical agents on the epidermal barrier and serve as a promising tool for optimizing the therapeutic options for patients with ichthyoses.
Acknowledgements
We would like to thank all children and adults with ichthyoses for participating in our study and Ms. Kirsten Kiekbusch from Selbsthilfe Ichthyose e.V. for her support and great cooperation. Open access funding enabled and organized by Projekt DEAL.

Data availability statement
The data that support the findings of this study are available from the corresponding author upon reasonable request.

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Appendix 1

Table A1 Continuous data

|                         | KPI n = 11 | ARCl n = 27 | NTS n = 7 | XRI n = 1 | Controls n = 10 | P-value |
|-------------------------|------------|-------------|-----------|-----------|----------------|---------|
| SCORAD (points)         | n = 40     | n = 11      | n = 27    | n = 1     | n = 10         | n.a.    | P = 0.111† |
| SCORAD adapted (points) | n = 40     | n = 11      | n = 27    | n = 1     | n = 10         | n.a.    | P = 0.987‡ |
| Erythema (points)       | n = 40     | n = 11      | n = 27    | n = 1     | n = 10         | n.a.    | P = 0.112‡ |
| Oozing (points)         | 0 [0, 1]   | 0 [0, 1]    | 0 [0, 0]  | 0         | 0              | 0       | P = 0.033† |
| Dryness (points)        | 2 [2, 2]   | 2 [2, 2]    | 2 [3, 2]  | 2         | 2              | 2       | P = 0.53†  |
| Itch intensity (points) | 2.9 [1, 1.4, 4.9] | 0.9 [2, 3, 5] | 0 [1, 4.9] | 2.5        | 0              | 0       | P = 0.439† |
| Sleep-loss (points)     | 0 [0, 2]   | 0 [0, 2]    | 0.2 [0, 3]| 0         | 0              | 0       | P = 0.457‡ |
| TEWL L [g/h/m²]         | 16.8 [13.2, 25.8] | 13.9 [10.8, 25.8] | 17.6 [13.9, 24.2] | 30.8 | 13.3          | P = 0.506‡ |
| TEWL NL [g/h/m²]        | 15.6 [11, 19.6] | 11.9 [9.2, 16] | 16.9 [12.8, 19.9] | 31.4 | 12            | 15.2 [11.5, 20.7] | P = 0.063‡ |
| Hydration L [arbitrary units] | 12 [7.7, 17] | 11.7 [7.3, 17] | 12.2 [7.6, 17.7] | 22.3 | 7.7          | < 0.001‡ | P = 0.63† |
### Table A1 Continued

| Ichthyoses n = 46 | KPI n = 11 | ARCI n = 27 | NTS n = 7 | XRI n = 1 | Controls n = 10 | P-value |
|------------------|----------|------------|---------|---------|----------------|--------|
| Hydration NL (arbitrary units) | n = 33 | n = 11 | n = 21 | n = 1 | n = 10 | P < 0.001‡ |
| nICLL L | n = 32 | n = 6 | n = 18 | n = 7 | n = 1 | P < 0.001‡ |
| nICLL NL (nm/1000 nm²) | 54.4 [38.8, 83] | 51.4 [40.4, 91.3] | 55.9 [39.2, 107.9] | 38.6 [33, 56] | 90.4 | P = 0.119§ |
| RoU | n = 32 | n = 6 | n = 18 | n = 7 | n = 1 | P < 0.001‡ |
| (arbitrary units) | 60.3 [42.7, 89.5] | 61.5 [49.7, 94.5] | 66.5 [42.9, 110.4] | 41 [33, 56] | 98.7 | P = 0.068§ |

Frequencies for categorial data by means of median with first and third quartile represented in square bracket.

ARCI, autosomal recessive congenital ichthyoses; KPI, keratinopathic ichthyoses; L lesional; n.a., not assessed.; nICLL, normalized intercellular lipid lamellae; NL, non-lesional; NTS, Netherton syndrome; RoU, ratio of undulation; SCORAD, severity scoring of atopic dermatitis; TEWL, transepidermal water loss; XRI, x-chromosomal-recessive ichthyosis.

††Results of non-parametric Mann–Whitney U-test ichthyosis group vs. control group.

*§Results of subsequent Bonferroni correction of ††KPI vs. ARCI vs. NTS.

**Results of non-parametric Kruskal–Wallis test KPI vs. ARCI vs. NTS.

### Table A2 Nominal data

| Ichthyoses n = 46 | KPI n = 11 | ARCI n = 27 | NTS n = 7 | XRI n = 1 | Controls n = 10 | P-value |
|------------------|----------|------------|---------|---------|----------------|--------|
| Age (years) | n = 39 | n = 11 | n = 26 | n = 1 | n = 10 | P < 0.001* |
| Sex | n = 46 | n = 11 | n = 27 | n = 7 | n = 1 | P < 1* |
| Female | 33 (72) | 11 (100) | 17 (63) | 5 (71) | 7 (70) | P = 0.05 |
| Male | 13 (28) | 0 | 10 (37) | 2 (29) | 1 | 3 (30) | 1† |
| Genetics | n = 40 | n = 11 | n = 27 | n = 1 | n = 1 | n.a. |
| Not yet | 1 (3) | 0 | 1 (4) | | | |
| Unknown | 9 (23) | 2 (18) | 9 (33) | | | |
| Identified | 4 (10) | 1 (9) | 2 (7) | | | |
| KRT 1 | 1 (3) | 8 (73) | | | | |
| KRT 10 | 8 (20) | | | | | |
| TGM 1 | 10 (25) | 10 (37) | | | | |
| CYP4F22 | 3 (8) | 3 (11) | | | | |
| ABCA12 | 1 (3) | 1 (4) | | | | |
| NIPAL4 | 1 (3) | 1 (4) | | | | |
| LEKTI | 1 (3) | 1 | | | | |
| STS | 1 (3) | 1 | | | | |
| Birth history | n = 40 | n = 11 | n = 27 | n = 1 | n = 1 | n.a. |
| Prematurity | 2 (5) | 0 | 1 (4) | 0 | 1 | P = 1* |
| Collodium baby | 6 (15) | 0 | 5 (19) | 0 | 1 | P = 0.295§ |
| Family history | n = 40 | n = 11 | n = 27 | n = 1 | n = 1 | n.a. |
| Unknown | 2 (5) | 0 | 2 (7) | | | |
| Blande | 22 (55) | 5 (46) | 16 (59) | 1 | | |
| Mother affected | 3 (8) | 3 (27) | 0 | | | |
| Daugther affected | 3 (8) | 3 (27) | 0 | | | |
| Brother affected | 3 (8) | 0 | 3 (11) | | | |
| Sister affected | 3 (8) | 0 | 3 (11) | | | |
| Parents conductors | 4 (10) | 0 | 3 (11) | 1 | | |
Table A2  Continued

| Ichthyoses n = 46 | KPI n = 11 | ARCI n = 27 | NTS n = 7 | XRI n = 1 | Controls n = 10 | P-value |
|------------------|-----------|-------------|-----------|-----------|----------------|---------|
| Previous condition | n = 40 | n = 11 | n = 27 | n = 1 | n = 1 | n = 10 | |
| Allergies | 8 (20) | 1 (9) | 6 (22) | 1 | 0 | 2 (20) | \( P = 1^a \) |
| Rhinoconjunctivitis | 3 (8) | 0 | 3 (11) | 0 | 0 | 4 (40) | \( P = 0.023^a \) |
| Atopy in childhood | 0 | 0 | 0 | 0 | 3 (30) | \( P = 0.006^a \) |
| Therapy | n = 40 | n = 11 | n = 26 | n = 1 | n = 1 | n.a. | |
| Balneotherapy | 36 (90) | 11 (100) | 23 (88) | 1 | 1 | | \( P = 0.673^b \) |
| + keratolytic† | 15 (38) | 3 (27) | 11 (42) | 1 | 0 | | | \( P = 0.628^b \) |
| + disinfectant | 3 (8) | 3 (27) | 0 | 0 | 0 | | | \( P = 0.02^b \) |
| + oil | 3 (8) | 1 (9) | 1 (4) | 1 | 0 | | | \( P = 0.653^b \) |
| + salt | 5 (13) | 3 (27) | 2 (7) | 0 | 0 | | | \( P = 0.275^b \) |
| Emollients‡ | 35 (88) | 10 (91) | 24 (89) | 0 | 1 | | | \( P = 1^b \) |
| + urea | 31 (78) | 9 (82) | 21 (78) | 0 | 1 | | | \( P = 1^b \) |
| + glycerin | 3 (8) | 0 | 3 (11) | 0 | 0 | | | \( P = 0.673^b \) |
| + lactat acid | 1 (3) | 0 | 1 (4) | 0 | 0 | | | \( P = 1^b \) |
| + dexpanderenol | 5 (13) | 1 (9) | 3 (11) | 1 | 0 | | | \( P = 0.674^b \) |
| disinfectant | 3 (8) | 2 (18) | 0 | 1 | 0 | | | \( P = 0.078^b \) |
| o. antihistamines | 2 (5) | 0 | 1 (4) | 1 | 0 | | | \( P = 1^b \) |
| o. retinoids | 5 (13) | 2 (18) | 3 (11) | 0 | 0 | | | \( P = 0.732^b \) |

Frequencies for nominal data in absolute and relative represented in round brackets numbers or median with first and third quartile represented in square bracket. P-values are results of the non-parametric \( \chi^2 \) test or Fisher’s exact test of *ichthyosis vs. control group* † KPI vs. ARCI ‡ KPI vs. ARCI vs. NTS. †, additive; ABCA, ATP-binding cassette; ARCI, autosomal recessive congenital ichthyoses; CYP, cytochrome P450; KPI, keratinopathic ichthyoses; KRT, keratine; LEKTI, lymphoepithelial-Kazal-type 5 inhibitor; n.a., not assessed; NIPAL, NIPA-like domain; NS, Netherton syndrome; o., oral; STS, steroid sulfa-tase; TGM, transglutaminase; XRI, x-chromosomal-recessive ichthyosis.

†Baking soda or starch.
‡Lipophilic base: basis DAC (n = 4), abitima (n = 4), unguentum cordes (n = 3), dexeryl (n = 3), excipial (n = 2), other.