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An IL-17-dominant immune profile is shared across the major orphan forms of ichthyosis

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Abstract

Background: Ichthyoses are rare, debilitating disorders associated with generalized scaling, erythema, and epidermal barrier impairment. Pathogenesis-based therapy is largely lacking, since the underlying molecular basis is poorly understood.

Objective: To characterize molecularly cutaneous inflammation and its correlation with clinical and barrier characteristics.

Methods: We analyzed biopsies from 21 genotyped ichthyosis patients (congenital ichthyosiform erythroderma, lamellar ichthyosis, epidermolytic ichthyosis, and Netherton syndrome) by immunohistochemistry and RT-PCR and compared them with healthy controls, and with atopic dermatitis (AD) and psoriasis patients. Clinical measures included a severity score for ichthyosis (IASI), which integrates erythema (IASI-E) and scaling (IASI-S), transepidermal water loss (TEWL), and pruritus.

Results: Ichthyosis samples showed increased epidermal hyperplasia (increased thickness and K16 expression) and T-cell and dendritic-cell infiltrates. Increases of general inflammatory (IL-2), innate (IL-1β), and some Th1/IFN (IFNγ) markers in ichthyosis were comparable to psoriasis or AD. TNFα levels in ichthyosis were elevated only in Netherton syndrome, but were much lower

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than in psoriasis and AD. Expression of Th2 cytokines (IL-13, IL-31) in ichthyoses was similar to controls. Most notable in ichthyosis was the striking induction of IL-17-related genes or markers synergistically induced by IL-17 and TNFα (IL-17A/C, IL-19, CXCL1, PI3, CCL20, IL36G; $p<0.05$), similar to psoriasis. IASI and IASI-E strongly correlated with IL-17A ($r=0.74$, $p<0.001$) and IL-17/TNF-synergistic/additive genes. These markers also significantly correlated with TEWL, suggesting a link between functional barrier defects and inflammation in ichthyosis.

Conclusion: Our data associates the shared ichthyosis immune fingerprinting to Th17/IL-23 polarization, and raises the possibility of IL-17-targeting strategies for the ichthyoses.

Clinical Implications: The link between Th17 pathway activation and clinical disease severity raises the possibility of a new therapeutic paradigm of targeted IL-17/IL-23 intervention for ichthyosis patients.

Capsule Summary: CIE, LI, EI and NS subtypes of ichthyosis are Th17-skewed. IL-17/TNF-synergistic/additive genes are most dominantly activated and significantly correlated with disease severity scores and functional barrier abnormalities (TEWL).

Keywords

Ichthyosis; inflammation; autosomal recessive congenital ichthyosis; congenital ichthyosiform erythroderma; lamellar ichthyosis; Netherton syndrome; epidermolytic ichthyosis; skin; IL-17; TNFα

Introduction

Ichthyoses are rare, genetically and clinically heterogeneous disorders with generalized skin scaling, thickening, and erythema.[1–6] Affected individuals have an extremely compromised quality of life because of disfigurement and accompanying itching, pain, and functional limitation.[7,8] The epidermal barrier is abnormal with defects in lipids, differentiation, and transepidermal water loss/TEWL.[9–11]

Treatment for ichthyosis is largely supportive and unsatisfactory. For more severely affected individuals, oral retinoids, vitamin A analogues, are often administered to improve the hyperkeratosis.[12–14] However, retinoids can worsen skin inflammation and pruritus, and have deleterious effects (hypertriglyceridemia, teratogenicity, hyperostosis),[15] limiting their use. Topical anti-inflammatory medications (i.e., steroids, calcineurin inhibitors) are often ineffective and easily absorbed systemically, restricting chronic use.[16,17] Thus, a huge unmet need exists for safe, more effective treatments which ideally will also target the erythema/inflammation.

Despite an improved understanding of the genetic basis,[1] the molecular mechanisms for various ichthyosis forms are poorly understood and predominantly based on culture and animal models.[18–28] These model systems chiefly focus on abnormal barrier function and lipid homeostasis, with attention paid to immune disturbances.[6,29,30] The limited data from ichthyosis patients primarily involves small numbers of individuals with Netherton syndrome/NS or the lamellar ichthyosis/LI form of autosomal recessive congenital ichthyosis/ARCI, and has explored a few cytokines in blood with little study in human skin.

[31–37] Blood analyses found inconsistent Th2 skewing[38] and increases in pro-inflammatory cytokines (tumor necrosis factor-alpha/TNFα, IL-1β, IL-2, IL-18).[39–41] Skin studies showed increased expression of TNFα and IL-1β in ARCI-LI,[34] and of protease-activated receptor 2/PAR2,[31] thymic stromal lymphopoietin/TSLP, TNFα, IL-8,[42] and the Th2 cytokine IL-33 in NS,[37] often coupled with increased expression of terminal differentiation products (i.e filaggrin/FLG, loricrin/LOR, involucrin/IVL), and lipid impairements.[31,34,36,37] A few human studies investigated changes in a limited array of barrier or immune markers with systemic treatments, including retinoids (n=11), anti-TNF (n=1), and oral corticosteroids combined with omalizumab (n=1) in ARCI-LI and NS patients, respectively.[32–34] Therapy-induced decreases in IL-1β, IL-8, TSLP, IL-5, and IL-17A were found in NS, while IL-1α and TNFα were decreased (non-significantly) in ARCI-LI.

To elucidate the basis for the cutaneous inflammation in ichthyosis and its correlation with clinical characteristics, we analyzed skin from 21 individuals with three of the more prevalent orphan ichthyoses: ARCI (LI and congenital ichthyosiform erythroderma/CIE forms), epidermolytic ichthyosis/EI, and NS. All subtypes showed Th17-skewing in skin, which correlated with disease severity. This Th17 profile most closely resembled that of psoriasis, in which IL-17 antagonism is highly effective in reversing the inflammation and epidermal pathology.[43–46] These data may lead to a new treatment paradigm targeting the Th17/IL-23 pathway in ichthyosis.

Methods

Patient characteristics

21 patients (ages 10–57 years) with ichthyosis and known mutations were enrolled (Tables 1–E1, Supplementary Materials in this journal's online repository/OR). Written IRB-approved consent was provided by subjects (≥12 years) and parents (<18 years). Demographic information, medical history, physical examination, clinical severity scores, pruritus (5-D Itch Scale and Itch Numerical Rating Scale/NRS), photography, and TEWL were captured. Few scoring instruments have been used for ichthyosis severity, and the only validated one (the Congenital Ichthyoses Severity Index/CISI) includes erythema and hyperkeratosis/scaling, but also alopecia (found in the minority of patients), fails to score potential differences in body regions, and is geared towards patient-reported assessment.[47] As a result we quantified severity using a tool that modifies the 5-point Likert CISI scale, eliminating alopecia and prorating based on body region and extent to create a composite score, similar to the Psoriasis Area and Severity Index/PASI.[48] This Ichthyosis Area and Severity Index/IASI measures severity of the erythema/IASI-E and scaling/IASI-S, adding them together to a total IASI score (Tables 1, E3; Supplementary Methods in OR). 4mm biopsies were collected and compared with normal, AD and psoriasis tissues previously published by our group.[49–53] Four samples of healthy adolescents were also included (Table E2) for appropriate comparisons with the younger ichthyosis cohort. Patient characteristics are presented in Tables 1, E1–E2.

Quantitative RT-PCR

RT-PCR was performed as described.[54,55] Expression values were normalized to human acidic ribosomal protein/hARP.

Immunohistochemistry

IHC was performed on frozen sections as described.[56] Antibodies are listed in Table E7. IHC data are shown in Table E8.

Statistical Analyses

hARP normalized RT-PCR expression values under the limit of detection/LOD were imputed as 20% of the minimum observed values (over LOD) and $\log_2$-transformed prior to analysis. No other missing value imputation method was performed and all available observations were included in analyses, which were performed using the statistical language R (www.R-project.org) and its available packages. Differences in expression values (in $\log_2$-scale), cell-counts, and clinical variables were assessed using linear models.

Unsupervised hierarchical clustering of variables or samples/patients was performed using Pearson correlation coefficient as a distance metric with the Mcquiity agglomeration algorithm. The results are represented as a heatmap with a dendrogram, and a tree or phylogram (using R package *ape*) (see extended statistics in OR).

Results

Demographics and clinical characteristics of ichthyosis subjects

21 individuals with 3 ichthyosis subtypes were included: 13 patients with two forms of ARCI (CIE, n=6; and LI, n=7), EI (n=5), or NS (n=3), ages ≥10 years, with known genetic mutations (Tables 1 and E1). ARCI patients are typically born as “collodion babies” (Fig. 1A),[57,58] and have the eventual phenotype ranging from large plate-like scales overlying variable erythema (LI) (Fig. 1B) to fine flaky scale and intense erythema (CIE) (Fig. 1C).[1,3] All LI patients had mutations in *TGM1*, encoding transglutaminase, which enables stratum corneum crosslinking;[59] CIE subjects had a range of mutated genes (Table E1), particularly encoding proteins of the hepoxilin pathway.[1,60,61] EI patients display erythema under warty scale (Fig. 1D); all our patients had *KRT10* mutations. NS, resulting from mutations in *SPINK5*,[62,63] encoding a protease inhibitor, ranges from milder erythema with unique scaling (ichthyosis linearis circumflexa) to generalized erythroderma (Fig. 1E–F, respectively). Normal skin from healthy individuals (≥10 years; n=16; Table E2) as well as lesional and non-lesional skin from moderate-to-severe adults with two common skin disorders, atopic dermatitis (AD, n=16; Fig. 1G) and psoriasis (n=10; Fig. 1H), were also included for appropriate comparisons with all polar cytokine pathways (Table 1).[49–53] Due to age differences between groups, with ichthyosis being the youngest cohort ($p<0.001$), all analyses were age-adjusted (detailed Statistics in OR; Tables 1, E1).

There were no significant differences in IASI or IASI-E scores among subtypes (Fig. 1I–K). However, LI and EI showed greater IASI-S in comparison with CIE ($p<0.01$ for LI, $p<0.05$ for EI) and NS ($p<0.05$ for LI) (Fig. 1J). Two pruritus scores were measured, the Itch

Numerical Rating Scale/NRS and the 5-D Itch Scale.[64] Since the two itch scales were highly correlated (Fig. E1), the 5-D Itch Scale was used for correlations. The 5-D Itch Scale was significantly higher ($p<0.05$) for NS compared to EI and CIE (Fig. 1L). The mean TEWL, a measure of barrier function, was also significantly greater in NS compared to EI and LI, and in CIE compared to EI subtype ($p<0.05$) (Table 1, Fig. 1M).

Increased hyperplasia and cellular infiltration characterize ichthyotic skin

Epidermal hyperplasia (as measured by epidermal thickness, mRNA and protein expressions of keratin 16/K16, a marker of epidermal proliferation) [65,66] was seen in all ichthyosis subtypes compared to controls, with the greatest increases observed in NS (Fig. 2A). K16 staining patterns in ichthyosis were comparable to those of lesional AD and psoriasis, characterized by widespread K16 staining (16/16 and 10/10, respectively) (except LI, with only 3/7 $K16^+$; Fig. 2B). The highest increases in epidermal thickness and K16 mRNA were seen in EI and NS (Fig. 2A–B, 2F–G). Significant increases in $CD3^+$ T-cell and $CD11c^+$ myeloid dendritic cells/DCs, DC-$LAMP^+$ DCs, and neutrophil $elastase^+$ neutrophils characterized all ichthyosis subtypes compared to controls, with greatest increases observed in NS (Fig. 2C–D, 2H–I, Fig. E2). The infiltrates in ichthyosis were comparable to those of highly inflammatory lesions from AD and psoriasis patients. In fact, NS had similar increases in neutrophils (vs. control skin) to psoriasis, considered a highly neutrophilic disease (Fig. E2).[53] Unlike AD and psoriasis, no significant increases in $CD1a^+$ Langerhans cells/LC (Fig. E2).

We also analyzed protein and mRNA expression of the keratinocyte differentiation markers (filaggrin/FLG, loricrin/LOR, periplakin/PPL), which are largely down-regulated in AD. [10,67–69] Unlike the continuous, clear expression of FLG in normal skin, AD lesions showed skipped and faint FLG expression in the upper layers of the epidermis, including the stratum corneum (Fig. 2E). Similar to psoriasis, most ichthyosis tissues, and particularly NS, showed increased and more intense expression of FLG, in the spinous and granular layers, compared with control skin. This was paralleled by significantly increased mRNA expression of FLG, LOR, and PPL, which was even higher in ichthyosis than psoriasis, but largely suppressed in AD compared to controls, as reported,[68,70] and consistent with reduced FLG immunostaining (Figs 2J, Fig. E3).

Ichthyotic skin shows a Th17-centered inflammation

To evaluate primary Th1, Th2, Th9, Th17, Th22 cytokines and some epidermal markers, which are often below detection levels on gene-arrays,[71] we performed qRT-PCR. We observed large increases in expression of general inflammatory (IL-2, IL-15) and some innate immune (IL-1β, IL-8) markers in ichthyosis compared with control skin (Figs. 3–4A, Fig. E3). These increases were comparable and even higher than those in AD and psoriasis. Interestingly, TNFα was up-regulated in AD and psoriasis compared to controls, but not in ichthyosis, although higher levels were seen in NS, as reported (Fig. 3–4A).[6,32,42] Expression of Th1-related markers (IFNγ, CXCL10, CXCL9) was also increased in ichthyosis compared to controls (Figs. 3–4A, Fig. E3). The expression of Th2 cytokines (IL-13, IL-31, IL-5, CCL17) was lower in ichthyosis than in AD, and largely similar to controls (Fig. 3–4A, Fig. E3). Some Th2 markers (CCL18, IL-10, CCL22) showed increases

in NS, but much smaller than in AD and comparable or even lower than in psoriasis (Figs. 3–4A, Fig. E3). IL-9/Th9 cytokine was not increased in ichthyosis compared with controls (Fig. 3).

In contrast, Th17/IL-23 pathway genes were significantly induced, including those previously reported as synergistically or additively regulated by IL-17 and TNFα (highlighted by green boxes in Figs. 3–4A, and E3).[72] IL-17A, p19 and p40 IL-23 subunits, IL-20, IL-23R, and IL-17-induced chemokines (i.e., HBD3) were significantly elevated, and up-regulation of IL-17/TNFα-synergistic/additive genes (IL-19, IL-17C, IL-36G/IL1F9, PI3, CCL20, DEFB4, S100A9) was particularly striking. NS showed the highest induction of Th17 pathway genes among ichthyosis subgroups, including the largest expression of IL-19, which is induced by both Th17 and Th2 cytokines, and in turn amplifies the IL-17 effects in keratinocytes (Figs. 3–4A).[73–77] Although not directly induced by TNFα, IL-19 is synergistically induced by IL-17 and TNFα.[72] Many IL-17-related factors, including those displaying a synergistic/additive effect with TNFα, showed comparable or even higher (IL-17C, CCL20, IL-36G) up-regulation in ichthyosis compared to lesional psoriasis (Figs. 3–4A). While IL-22/Th22 was only mildly elevated in NS and CIE, the S100As (S100A8/9/12), induced by both IL-17 and IL-22,[78] showed significant increases in ichthyosis versus controls. A cluster of IL-17-related and IL-17/TNFα-synergistic/additive genes (IL-17A/C, lipocalin-2/LCN2, S100A8/12, IL-36G, IL-20, PI3) was up-regulated in ichthyosis to a similar extent as in psoriasis (and much higher than AD; highlighted green box in Fig. 4A). All RT-PCR values and comparisons are listed in Table E4.

To further evaluate how ichthyosis profiles relate phenotypically to psoriasis, we performed an unsupervised hierarchical clustering of ichthyosis, AD, psoriasis, and control skin using expression profiles of all markers evaluated by qRT-PCR. Results are represented as a phylogenetic tree (Fig. 4B) showing the tight clustering of ichthyosis and psoriasis lesions, while controls and AD are much farther. Of note, ichthyosis tissues did not sub-cluster by subtype.

Erythema and disease severity highly correlate with IL-17 activation in ichthyosis

To determine how clinical severity, as measured by total IASI and its sub-scores, IASI-E (erythema/inflammation) and IASI-S (scaling), is linked to individual cellular or molecular markers, we used Pearson correlation-coefficients. Markers showing the highest correlations with total IASI score included IASI-E (r=0.74), IL-17A (r=0.57), IL-17-related markers (i.e., PI3, r=0.61) and the proliferation marker K16 (r=0.49) ($p<0.03$; Fig. 5A, Table E5). Highly significant correlations were found between IASI-E and IL-17A (r=0.74) and IL-17-related or IL-17/TNF-synergistic genes (CXCL1, PI3, IL-36G, S100As, IL-23p19, DEFB4, LCN2; $P<0.005$). Significant correlations were also noted between IASI-E and K16 and other immune (IL-1β) or cellular (DC-LAMP$^+$) markers (Fig. 5B, Table E5). IASI-S was significantly correlated only with IASI. The 5-D Itch scale showed few, non-significant correlations. TEWL showed significant correlations with many IL-17-related markers (i.e IL-17A/IL-17-C, LCN2, CXCL1) and with TNFα (Fig. 5C and Table E5).

To evaluate how different clinical scores (IASI, IASI-E, IASI-S, pruritus, TEWL) relate to biomarkers, we performed unsupervised hierarchical clustering of clinical scores (*blue*), cell

counts, thickness measurements, and mRNA expression (*black;* and *green*: IL-17/TNF-synergistic/additive genes) for all ichthyosis subtypes using Pearson correlation as a similarity metric and Mcquitty as an agglomeration algorithm. A graphic representation of the distance between variables is presented as a phylogenetic tree, with closer distances reflecting higher correlations (Fig. 6A). A tight cluster was found between IASI-E and IL-17-induced or IL-17/TNFα-synergistically modulated markers (IL-17A, CXCL1, DEFB4, PI3, etc), supporting the link between IL-17 activation and ichthyosis erythema. In proximity to this cluster are two clusters of IL-17/IL-23/TNFα-related genes (IL-22, IL-12/23p40 and IL-17C, IL-20; Fig. 6A). TEWL clustered with IL-22, TNFα and close to IL-17 markers, and the thickness measure clustered with IASI-S and close to terminal differentiation markers (LOR, LOR, PPL), reflecting a possible link between barrier and immune measures. The 5-D Itch Scale closely clustered with Th2 markers, including IL-13, IL-5, the itch cytokine IL-31,[79,80] and CCL26. Markers of T-cells ($CD3^+$), DCs ($CD11c^+$, $DC\text{-}LAMP^+$), and neutrophils clustered together, and in proximity to a large cluster of IL-17/IL-23-related and other immune genes (Fig. 6A).

These data are also presented as a heatmap showing positive (*red*) or negative (*blue*) correlations of all molecular, cellular measures, and clinical measures in ichthyosis patients (Fig. 6B), with color intensity reflecting the correlation's strength. A green box shows the associations of IASI-E with IL-17A and other IL-17-related genes (that clustered together in the phylogenetic tree). Associations with scaling/thickness, TEWL, and 5-D Itch Scale are highlighted by pink, brown and grey boxes, respectively. Individual correlations with clinical scores are in Table E5.

Discussion

The ichthyoses are rare life-altering genetic disorders, characterized by scaling, epidermal thickening, and erythema.[1,5] Available treatments for ichthyosis (primarily oral retinoids) are unsatisfactory, lack specificity, and are associated with potential side effects. These treatments are primarily focused on reducing thickening and scaling, without addressing the erythema or inflammatory component.[12,15]

Few studies have evaluated the role of immune dysregulation in ichthyosis patients[32–34] with the underlying molecular basis predominantly based on limited data from in vitro and animal models.[6,18–30] These models observed pro-inflammatory signals, with increases in cytokines (IL-1, TNFα) and chemokines (S100As, CXCL1, TSLP, PAR2), and parallel epidermal hyperplasia (increases in K16, K6B) and abnormalities in differentiation (LOR, FLG) and lipid genes.[21,23,29,30,81] Mouse models of NS showed diverse cytokine activation with increases in innate, Th2, Th17 and Th22 cytokines (IL-1β, TNFα, IL-4, Il-13, IL-17, IL-22) and corresponding chemokines (TSLP, CCL17, CXCL1, CCL20, S100A8/9).[19] Moreover, inhibition of inflammation in model systems, using IL-37b overexpression and IL-1 blockade, considerably improved the epidermal phenotype, including the hyperplasia and aberrant differentiation.[21,30] The few investigations with human ichthyotic skin focused primarily on barrier alterations (hyperplasia, premature expression of terminal differentiation products and lipid defects).[31–37] Selected polar cytokines (i.e IFNγ, TNFα, IL-17, IL-8, IL-23, IL-9, IL-4, IL-5) have been found to be increased in keratinocytes and/or peripheral

blood from ichthyosis patients[38–41] and immune-modulators (retinoids, biologics, oral prednisone) decreased the expression of some of these cytokines (i.e IL-17, TNFα).[32–34]

The erythema, hyperkeratosis, and compromised barrier of ichthyosis are shared features with two common skin disorders, psoriasis and AD. In these diseases, inflammatory responses play an important role in disease progression.[44,46,50–52,82–86] Advances in understanding pathogenesis have translated into rapid development of cytokine-targeted therapeutics, which reverse the clinical inflammation but also the epidermal disease phenotype.[82,84–94] Pathogenesis-based therapies are available for psoriasis based on its Th17/IL-23-centered activation. TNFα has been functionally linked to the Th17/IL-23 pathway, and TNF antagonists are highly effective for psoriasis.[72,95] Furthermore, psoriasis treatment with etanercept, an anti-TNF, suppresses genes that are synergistically induced by IL-17 and TNF to a greater extent than TNFα-regulated genes alone.[72]

This is the first comprehensive molecular profiling of ichthyosis subtypes. Since ichthyoses share clinical and histological characteristics with AD and psoriasis (i.e., inflammation, epidermal hyperplasia and compromised barrier), we also compared the cutaneous signatures of major ichthyosis subgroups (ARCI-CIE, ARCI-LI, EI, and NS) with lesional and non-lesional skin from moderate-to-severe AD and psoriasis patients, as well as skin from healthy volunteers. This approach allowed determination of cytokine pathway up-regulation in ichthyosis and comparison with AD (primarily Th2-driven) and psoriasis (primarily Th17–23 driven). Our data show that all ichthyoses share impressive Th17/IL-23-skewing in skin. Similar to psoriasis, particularly large increases were observed in IL-17/TNF-synergistic/additive markers (IL-19, IL-17C, IL-36G, PI3, S100A12, CCL20), despite non-significant TNFα modulation. IL-36G has been reported to amplify TNFα and IL-17 pathways in psoriasis, and to accurately differentiate psoriasis from AD lesions.[96] The induction of genes modulated by IL-17 alone or IL-17 and TNFα together was largely comparable to psoriasis,[97,98] perhaps leading to tight clustering of ichthyoses and psoriasis samples. This was unexpected, given the pruritic nature of ichthyoses (especially CIE, NS) and their clinical resemblance to AD.[99] However, mRNAs of Th2 markers (e.g., IL-5, IL-13, IL-31, CCL17, CCL26) in all ichthyosis subsets were surprisingly low. The only exception was NS, in which some Th2 markers (CCL18, CCL22) were elevated. The concomitant increases of Th17- and Th2-related markers in NS might also contribute to the large increases in IL-19 in this subtype, given that Th17, but also Th2 cytokines can induce IL-19.[73–77] IL-19 induces epidermal hyperplasia and S100As,[73–77] which were highest in NS. Interestingly, although mouse NS models demonstrate increased Th2/Th17 responses,[6,19,20] Th2 inhibition through PAR2/TSLP suppression did not improve cutaneous inflammation.[29] Expression of Th1 markers varied in different ichthyoses, but was mostly lower than in AD and psoriasis. Increases in innate markers (IL-1β, IL-8) were also seen in ichthyoses and largely comparable with those in AD and psoriasis.

Importantly, the significant elevations in IL-17- and IL-17/TNF-modulated genes are strongly correlated with clinical severity. IASI, and particularly the erythema subscore/IASI-E was highly correlated with IL-17A and IL-17/TNF-regulated genes (CXCL1, IL-36G). Significant correlations were also found between IASI and IASI-E and epidermal hyperplasia, as measured by K16.[65,100] While in AD and psoriasis, epidermal hyperplasia is

linked to IL-22, IL-22 activation was far lower in ichthyosis than AD or psoriasis. Other hyperplasia-inducing IL-20 family cytokines (i.e., IL-19) might contribute to increased epidermal thickness in ichthyosis.[101–104]

The ichthyoses are recognized as having significant epidermal abnormalities,[66,70,105,106] including epidermal hyperplasia, higher TEWL, and lipid and differentiation abnormalities.[1,3] Hyperplasia and differentiation abnormalities, particularly in ARCI and NS subtypes, are similar to psoriasis, with hyperplastic epidermis and largely increased expression of differentiation proteins (LOR, FLG, PPL) in the upper epidermis.[31,42,107] We too found higher expression of these markers in ichthyosis and psoriasis, but much reduced expression in AD. The significant correlations between TEWL with IL-17A and IL-17/TNF-regulated genes (IL-17C, CXCL1, LCN2, IL-36G) may link the immune activation and functional barrier abnormalities in ichthyosis. Finally, similar to psoriasis, ichthyosis patients are able to mount significant IL-17-induced antimicrobial peptide/AMP responses, as shown by high expression of LL37, DEFB4B, HBD3, and CCL20. AMPs were recently shown[108,109] to up-regulate tight junctions and keratinocyte differentiation, and may further explain increases in these products in ichthyosis and psoriasis vs. AD patients. Indeed, although *Staphylococcus aureus* infections occur more often in ichthyosis than in psoriasis, their frequency is considerably less than in severe AD.[97,110]

Targeting the Th17/IL23 pathway in psoriasis and Th2 pathway in AD has not only suppressed lesional inflammation, but has also improved the epidermal pathology.[44,50,52,68,83–85,92,111,112] Future studies using IL-17/IL-23 or TNF-targeting strategies are needed to determine the contribution of immune activation in ichthyosis, and whether the barrier abnormalities can be ameliorated when inflammation is reduced. Interestingly, pruritus, although described in >70% of subjects, was not linked to IL-17A-related markers, and clustered with Th2 cytokines, including IL-31, a cytokine correlated with itch in AD.[79,113,114] Whether anti-IL-17/IL-23/TNF-targeting strategies could also reduce itch also remains a question.

Limitations of our data include the small sample size, reflecting the rarity of ichthyoses. Nevertheless, a large and significant effect was observed for IL-17-modulated or IL-17/TNF-synergistically regulated markers and their association with disease severity. Also, although 25% of controls were children ≥10 years old, the age difference between ichthyosis and healthy subjects was statistically significant. However, IL-17 expression increases with age in healthy skin,[115,116] suggesting that our results might actually underestimate the increased Th17 activation in ichthyosis. Furthermore, our AD and psoriasis samples were obtained from individuals ≥18 years old, although 8/21 of ichthyosis subjects were <18yo. Although the adolescent skin phenotype in AD and psoriasis is commonly considered close to adults, there are no studies comparing the two. Thus, for proper comparisons, all our analyses were age-adjusted. Future studies should address the effect of age on the observed differences.

Our data links Th17/IL-23 pathway and IL-17/TNF synergistic interactions with ichthyosis severity and inflammation, providing evidence that ichthyosis more closely resembles psoriasis in its immune profile. The linkage between immune alterations and functional barrier abnormalities in ichthyoses potentially suggests a similar model to psoriasis and AD,

in which increased cytokine production perpetuates the barrier alterations. These results imply that psoriasis therapeutics might be applicable for ichthyosis patients. One NS patient demonstrated clinical improvement and reduction in levels of IL-17 after administration of infliximab (anti-TNFα used to treat psoriasis).[32,117,118] IL-17/IL-23-targeting strategies,[92,111,119,120] have been shown to be more effective than TNFα inhibitors in psoriasis, dramatically improving PASI scores.[90,121] We propose that specific IL-17/IL-23-targeting might establish a novel shared treatment paradigm for the ichthyoses, and will further elucidate the underlying molecular basis and role of IL-17 activation.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations:

AD	Atopic dermatitis
AMP	Antimicrobial peptides
ARCI	Autosomal recessive congenital ichthyosis
CIE	Congenital ichthyosis erythroderma
CISI	Congenital ichthyoses severity index
DC	Dendritic cell
EI	Epidermolytic ichthyosis
FCH	Fold change
FLG	Filaggrin

hARP	Human acidic ribosomal protein
IASI	Ichthyosis area severity index
IASI-E	Ichthyosis area severity index-Erythema
IASI-S	Ichthyosis area severity index-Scaling
IHC	Immunohistochemistry
K16	Keratin 16
LC	Langerhans cells
LCN2	lipocalin-2
LEKI	Lympho-epithelial Kazal-type-related inhibitor
LI	Lamellar ichthyosis
LOD	Limit of detection
LOR	Loricrin
NRS	Numerical Rating Scale
NS	Netherton syndrome
PAR2	Protease-activated receptor 2
PASI	Psoriasis area and severity index
PPL	Periplakin
qRT-PCR	Quantitative real-time PCR
TEWL	Transepidermal water loss
TSLP	Thymic stromal lymphopoietin

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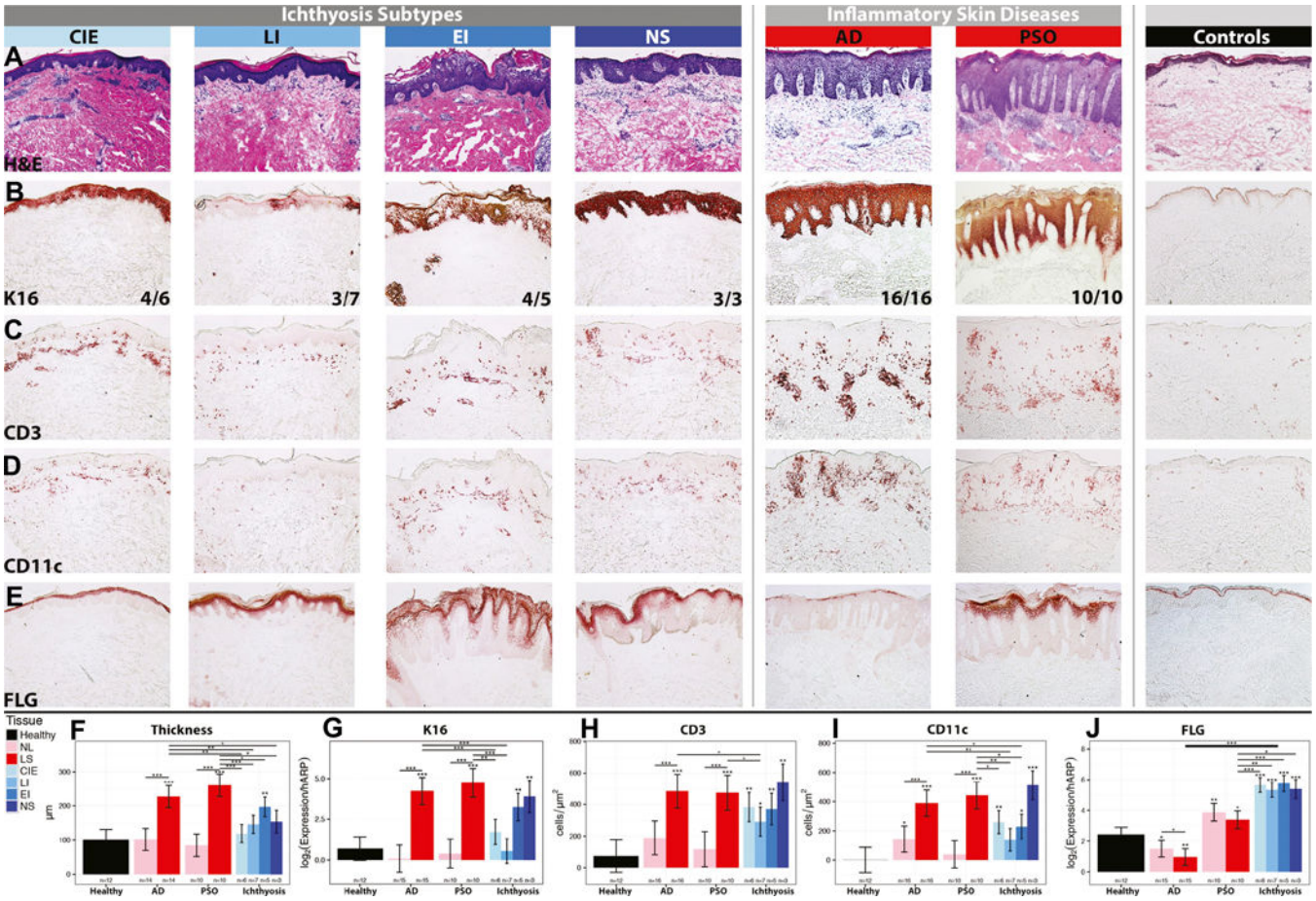

Figure 1.

Representative clinical pictures of the collodion-baby phenotype **(A)**, lamellar ichthyosis/LI **(B)**, congenital ichthyosis erythroderma/CIE **(C)**, epidermolytic ichthyosis/EI **(D)**, and Netherton syndrome/NS **(E-F)**. They share varying degrees of erythema and scaling, as in two common inflammatory skin diseases, atopic dermatitis/AD **(G)** and psoriasis **(H)**. **(I-M)** Clinical severity scores by subtypes of ichthyosis. +p<0.1, *p<0.05, **p<0.01.

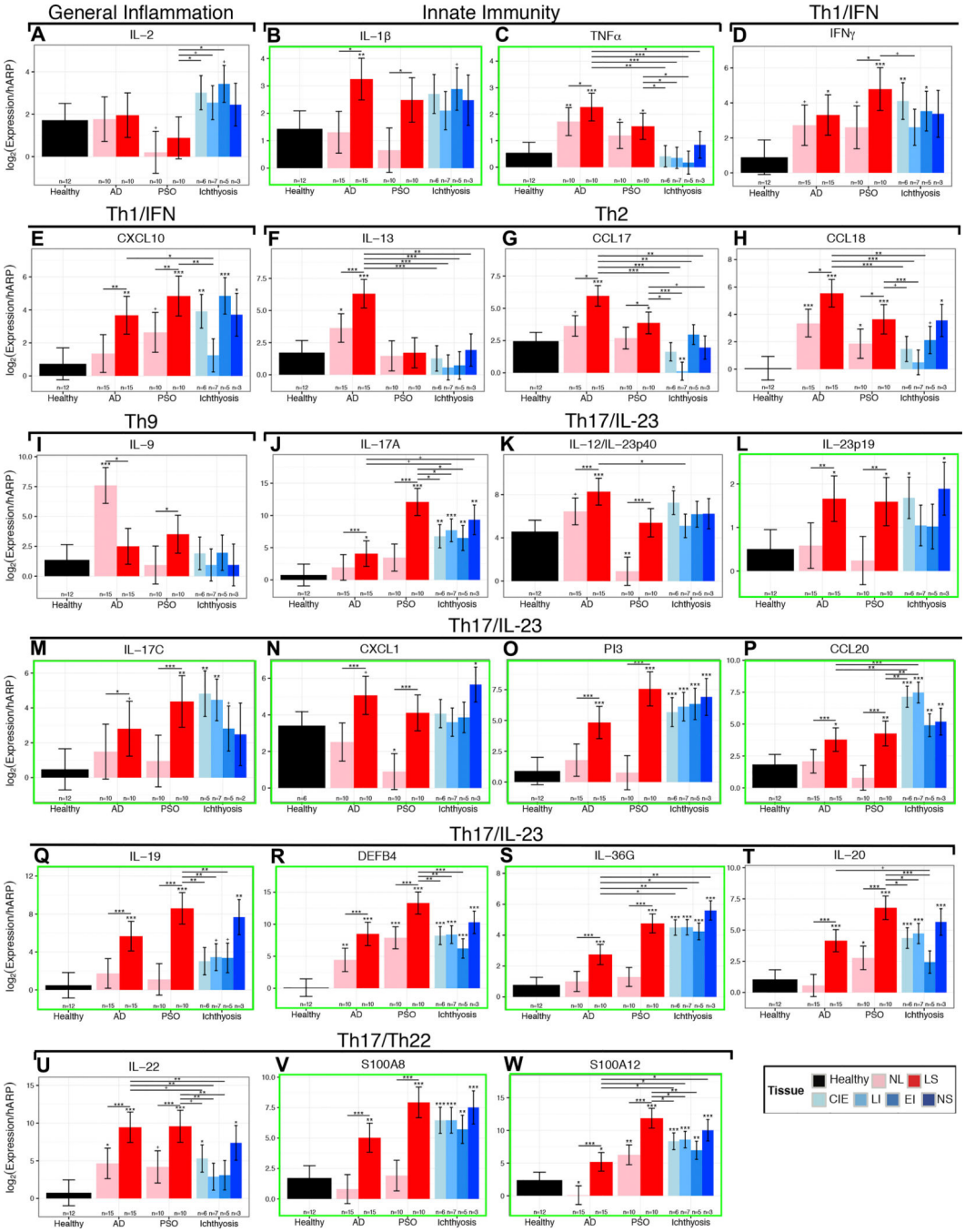

Figure 2.

Representative staining in ichthyoses, AD, psoriasis/PSO and controls using **(A)** hematoxylin-eosin/H&E, **(B)** K16 with fractions of positive samples, **(C)** $CD3^+$ T-cells, **(D)** $CD11c^+$ DC, and **(E)** filaggrin/FLG. Quantification of **(F)** epidermal thickness, **(G)** K16 mRNA, **(H-I)** $CD3^+$ and $CD11c^+$ cells and **(J)** filaggrin/FLG mRNA. mRNA-log_2 values were adjusted to hARP. Mean±SEM. Controls comparisons: stars above bars. *p<0.05, **p<0.01, ***p<0.001. LS: lesional; NL: non-lesional.

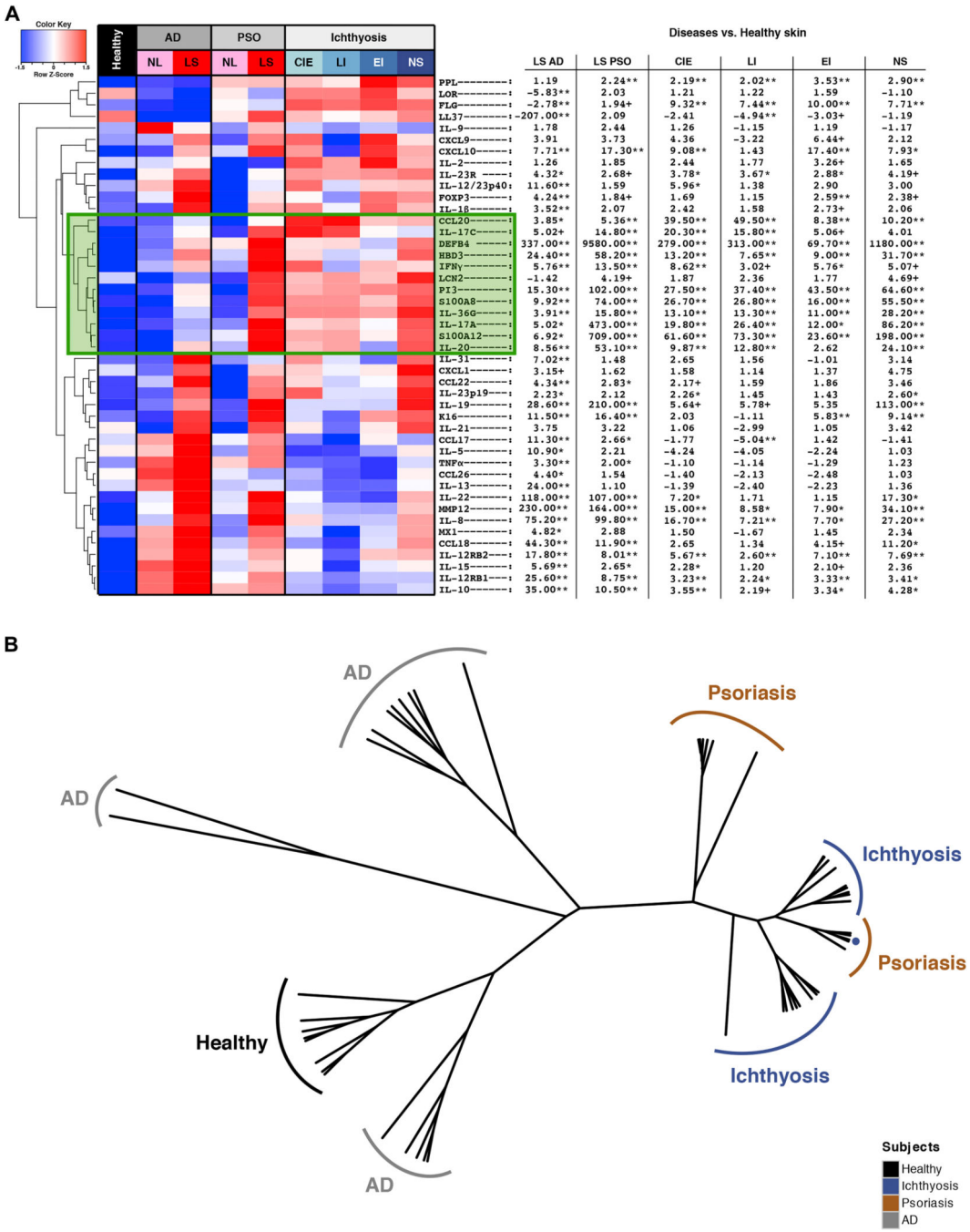

Figure 3.

Comparison of immune markers in ichthyosis subtypes, AD, psoriasis and controls using RT-PCR **(A-W)**. mRNA $\log_2$ values were adjusted to hARP expression levels. Stars without bars denote comparison to controls. Stars above bars denote p-values with comparators defined by the bar. LSmean ($\log_2$ expression/hARP) ± SEM. +p<0.1, *p<0.05, **p<0.01, ***p<0.001.

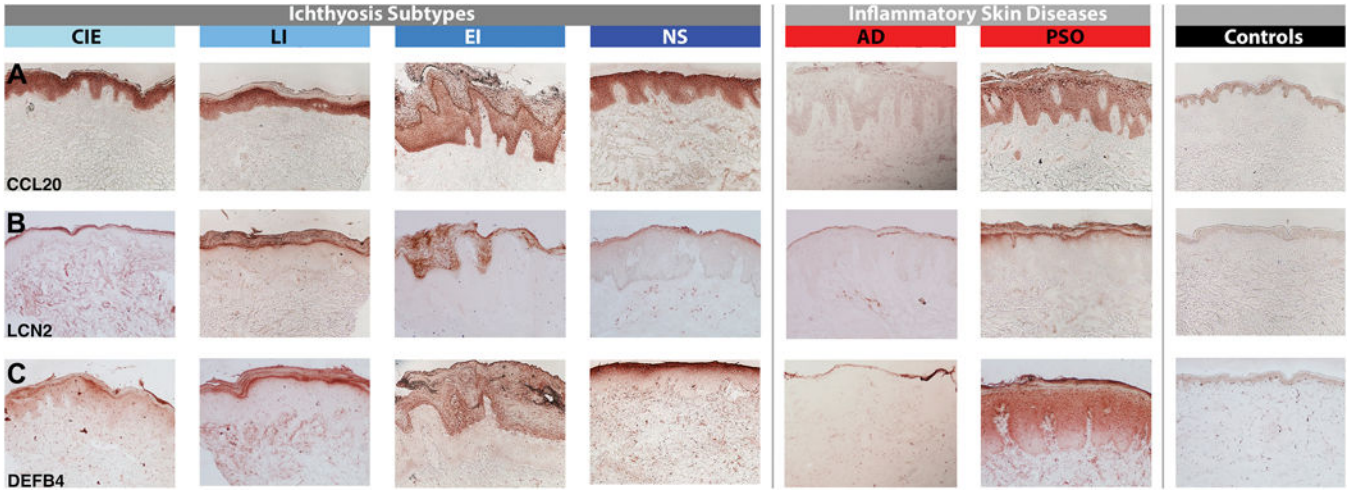

Figure 4.

Unsupervised hierarchical clustering of mRNA expression in AD, psoriasis, ichthyosis, and controls **(A)** as a heatmap with fold changes/FCHs between diseases and healthy skin. Green box: Cluster of upregulated IL-17-related genes in ichthyosis and psoriasis. +$p<0.1$, *$p<0.05$, **$p<0.01$. *red*, up-regulation; *blue*, down-regulation. **(B)** Unsupervised clustering of samples (phylogenetic tree) based on expression profiles of 45 immune/barrier markers; Distance: Pearson correlation, agglomeration: average.

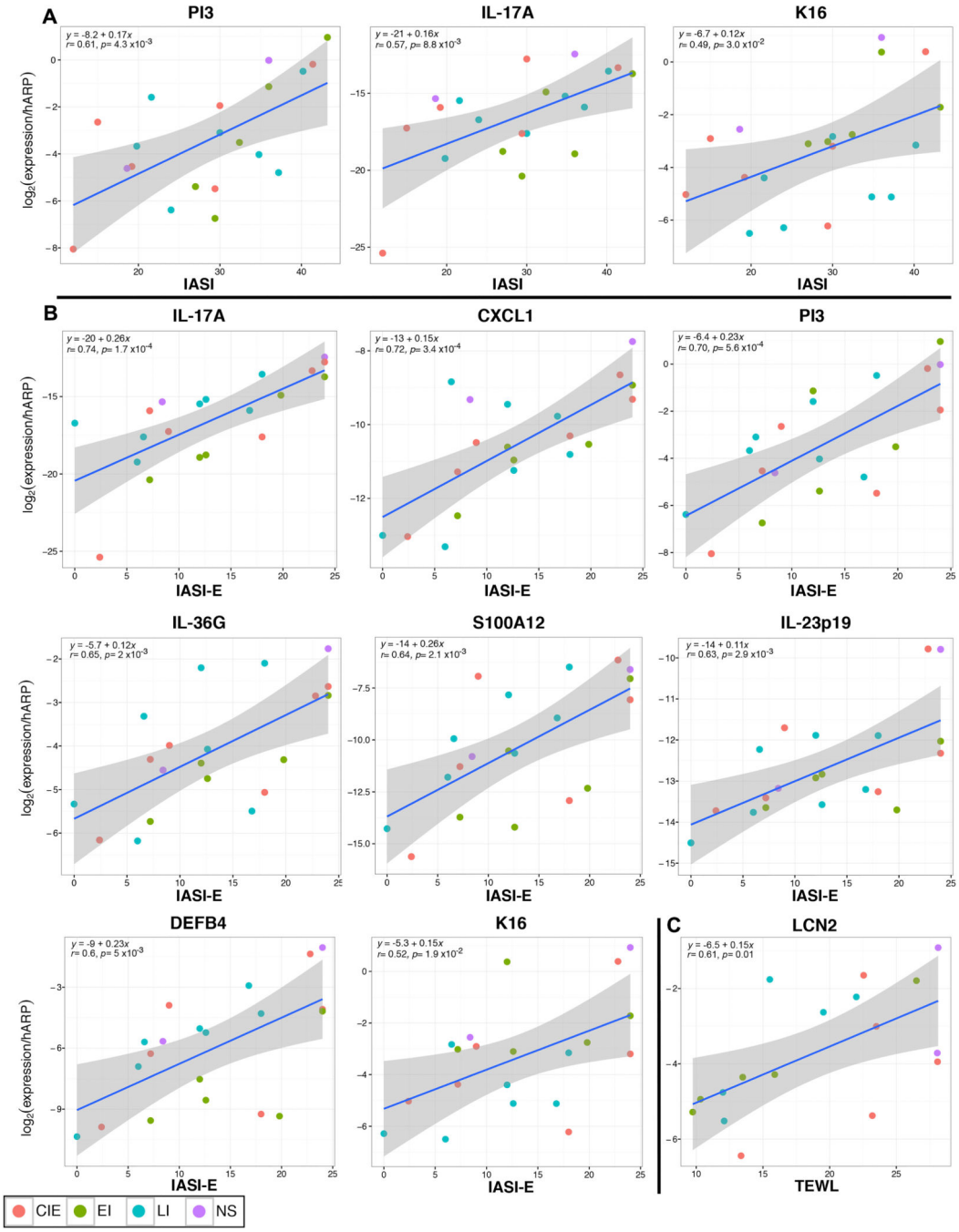

Figure 5.

Pearson correlation plots of the mRNA gene expression that correlated highest with **(A)** overall clinical severity score (IASI), **(B)** erythema severity subscore (IASI-E), and **(C)** TEWL in ichthyosis subtypes. *r* = Pearson correlation-coefficient with associated p value (*p*). *y* = equation for linear regression (blue line) with its confidence interval (smoothed confidence interval in grey).

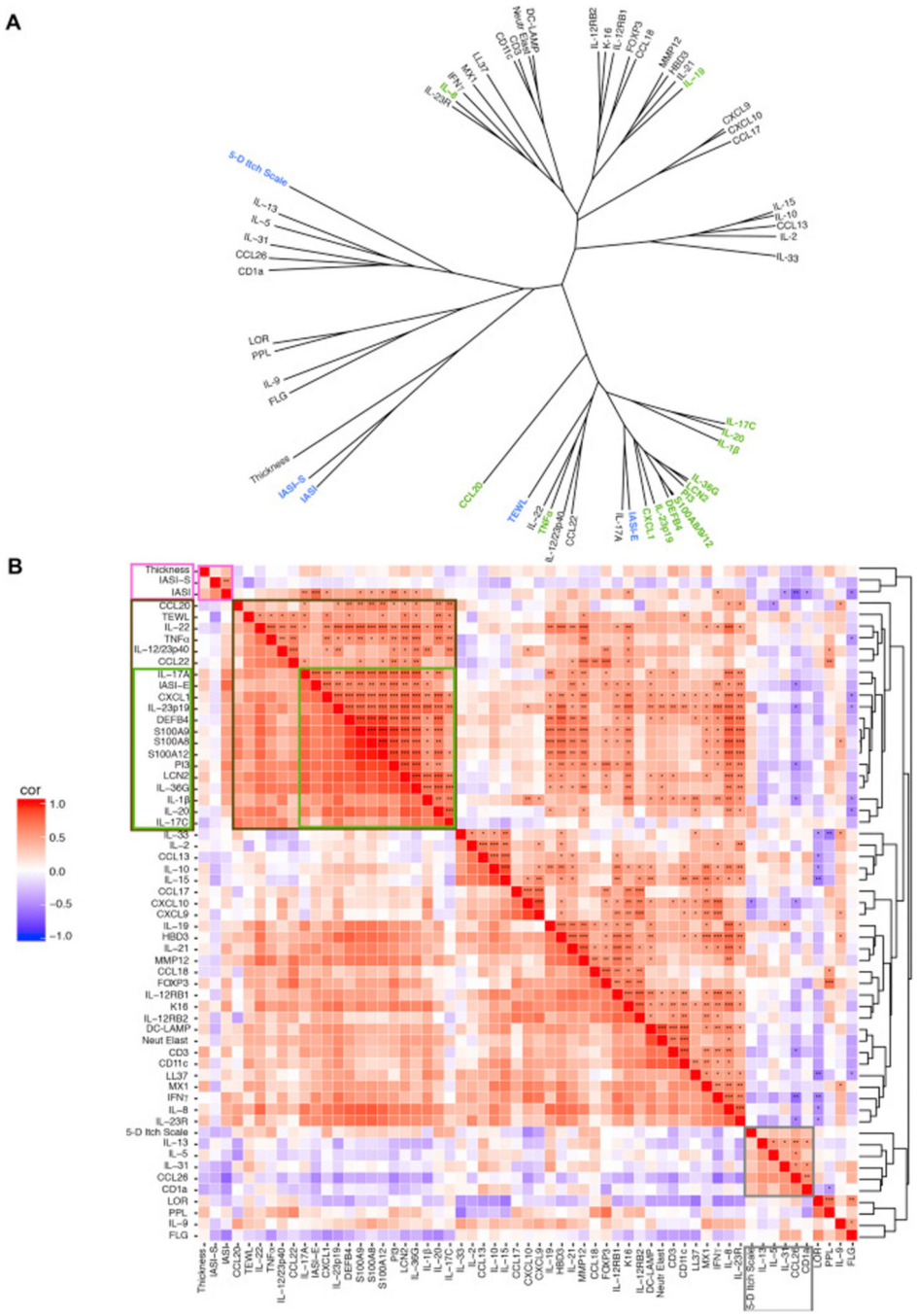

Figure 6.

Correlation matrix of all ichthyosis measurements. **(A)** Unsupervised hierarchical clustering of clinical (*blue*), with barrier/immune markers *(black)*, including IL-17-synergistic/additive genes *(green)*. Distance: Pearson correlation, algorithm: Mcquitty agglomeration. **(B)** Correlation heatmap. Pink box: correlations with IASI score. Brown box: the most significant cluster of IL-17- synergistic/additive genes, with green box highlighting the

IASI-E sub-cluster. Grey box: pruritus correlations. *red*, positive correlations; *blue*, negative correlations. *p<0.05, **p<0.01, ***p<0.001.

Table 1.

Patient demographics and clinical severity scores.

A. Characteristics of subjects with different ichthyosis subtypes.						
Characteristic	**Parameter**	**CIE (n = 6)**	**LI (n = 7)**	**EI (n = 5)**	**NS (n = 3)**	**P-value**
Age (years)	**Mean** (±SD)	26.4 ± 14.7	30.4 ± 14.8	27.6 ± 19.2	18.2 ± 5.3	0.718
	Median [age range]	23.2 [10.8 – 45]	28.0 [10.8 – 57]	17.9 [11.6 – 55]	19.2 [12.5 – 23]	
Gender	**Female**	3	6	3	1	0.511
	Male	3	1	2	2	
Race	**White**	5	5	5	2	0.695
	Black	0	1	0	1	
	Asian	1	0	0	0	
	Hispanic	0	1	0	0	
Disease Severity Scores	**IASI** (±SD)	24.5 ± 11.1	29.7 ± 8.0	33.6 ± 6.3	27.3 ± 12.3	0.437
	IASI-E (±SD)	13.9 ± 8.9	10.3 ± 6.4	15.1 ± 6.7	16.2 ± 11.0	0.652
	IASI-S (±SD)	10.6 ± 4.7	19.4 ± 5.5	18.5 ± 4.9	11.1 ± 1.3	0.017
	TEWL ($g/m^2/hr$, ±SD)	22.1 ± 5.4	16.2 ± 4.5	15.2 ± 6.8	28.1 ± 0.0	0.04
	5-D Itch Scale (±SD)	11.2 ± 3.1	13.8 ± 5.8	10.2 ± 3.1	17.0 ± 7.1	0.268

AD: atopic dermatitis; CIE: congenital ichthyosiform erythroderma; EI: erythrodermic ichthyosis; NS: Netherton syndrome; IASI: ichthyosis area severity index (with E: erythema; S: scaling); LI: lamellar ichthyosis; LS: lesionai; N/A: not applicable. NL: non- lesional; PASI: psoriasis area severity index; PSO: psoriasis; SCORAD: scoring atopic dermatitis; SD: standard deviation; TEWL: transepidermal water loss.

Table 2.

Comparison of ichthyosis patients vs. healthy controls and subjects with AD or psoriasis.

Comparison of ichthyosis patients vs. healthy controls and subjects with AD or psoriasis.							
Characteristic	**Parameter**	**Controls (n=16)**	**Ichthyosis (n=21; LS=21)**	**P-value Controls vs. Ichthyosis**	**AD (n=16; LS=16; NL=16)**	**PSO (n=10; LS=10; NL=10)**	**P-value All groups**
Age (years)	**Mean** (±SD)	38.7 ± 17.1	26.8 ± 14.6	0.033	52.8 ± 13.1	51.3 ± 11.0	< 0.001
	Median [age range]	44.5 [10.6–57]	23.0 [10.8–57]		49.5 [33–73]	54.0 [30–64]	
Gender	**Female**	7	13	0.444	8	4	0.609
	Male	9	8		8	6	
Race	**White**	13	17	0.269	16	10	0.201
	Black	0	2		0	0	
	Asian	0	1		0	0	
	Hispanic	3	1		0	0	
Disease Severity Scores			**IASI**		**SCORAD**	**PASI**	
	Mean (±SD)	N/A	28.9 ± 9.0		56.6 ± 10.7	20.3 ± 15.4	
	Median [IQR]	N/A	29.7 [21.2 – 36.0]		55.0 [51.5 – 63.0]	14.2 [12.1 – 21.2]	

AD: atopic dermatitis; CIE: congenital ichthyosiform erythroderma; EI: erythrodermic ichthyosis; NS: Netherton syndrome; IASI: ichthyosis area severity index (with E: erythema; S: scaling); LI: lamellar ichthyosis; LS: lesionai; N/A: not applicable. NL: non- lesional; PASI: psoriasis area severity index; PSO: psoriasis; SCORAD: scoring atopic dermatitis; SD: standard deviation; TEWL: transepidermal water loss.