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

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

Objective: We sought to characterize molecularly cutaneous inflammation and its correlation with clinical and barrier characteristics.

Methods: We analyzed biopsy specimens from 21 genotyped patients with ichthyosis (congenital ichthyosiform erythroderma, n = 6; lamellar ichthyosis, n = 7; epidermolytic ichthyosis, n = 5; and Netherton syndrome, n = 3) using immunohistochemistry and RT-PCR and compared them with specimens from healthy control subjects, patients with atopic dermatitis (AD), and patients with psoriasis. Clinical measures included the Ichthyosis Area Severity Index (IASI), which integrates erythema (IASI-E) and scaling (IASI-S); transepidermal water loss; and pruritus.

Results: Ichthyosis samples showed increased epidermal hyperplasia (increased thickness and keratin 16 expression) and T-cell and dendritic cell infiltrates. Increases of general inflammatory (IL-2), innate (IL-1β), and some T_H1/interferon (IFN-γ) markers in patients with ichthyosis were comparable with those in patients with psoriasis or AD. TNF-α levels in patients with ichthyosis were increased only in those with Netherton syndrome but were much lower than in patients with psoriasis and those with AD. Expression of T_H2 cytokines (IL-13 and IL-31) was similar to that seen in control subjects. The striking induction of IL-17–related genes or markers synergistically induced by IL-17 and TNF-α (IL-17A/C, IL-19, CXCL1, PI3, CCL20, and IL36G; $P < .05$) in patients with ichthyosis was similar to that seen in patients with psoriasis. IASI and IASI-E scores strongly correlated with IL-17A ($r = 0.74$, $P < .001$) and IL-17/TNF–synergistic/additive gene expression. These markers also significantly correlated with transepidermal water loss, suggesting a link between the barrier defect and inflammation in patients with ichthyosis.

Conclusion: Our data associate a shared T_H17/IL-23 immune fingerprint with the major orphan forms of ichthyosis and raise the possibility of IL-17–targeting strategies. (J Allergy Clin Immunol 2016;■■■:■■■-■■■.)

Key words: *Epidermis, ichthyosis, inflammation, autosomal recessive congenital ichthyosis, congenital ichthyosiform erythroderma, lamellar ichthyosis, Netherton syndrome, epidermolytic ichthyosis, skin, IL-17, TNF-α*

Ichthyoses are genetically and clinically heterogeneous disorders with generalized skin scaling, thickening, and erythema. Other than ichthyosis vulgaris and recessive X-linked ichthyosis subtypes,[1-7] the ichthyoses each occur in less than 1:100,000 persons. Affected subjects have an extremely compromised quality of life because of disfigurement and the accompanying itching, pain, and functional limitation.[8,9] The epidermal barrier is abnormal, with defects in lipids and differentiation resulting in increased transepidermal water loss (TEWL).[10-12]

Treatment for ichthyosis is largely supportive and unsatisfactory. For more severely affected subjects, oral retinoids, vitamin A analogues, are often administered to improve the hyperkeratosis.[13-15] However, retinoids can worsen skin inflammation and pruritus and have deleterious effects (hypertriglyceridemia, teratogenicity, and hyperostosis),[16] limiting their use. Topical anti-inflammatory medications (ie, steroids and calcineurin inhibitors) are often ineffective and easily absorbed systemically, restricting chronic use.[17,18] Thus a huge unmet need exists for safe and more effective treatments that will ideally also target the erythema/inflammation.

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Abbreviations used
AD: Atopic dermatitis
AMP: Antimicrobial peptide
ARCI: Autosomal recessive congenital ichthyosis
CIE: Congenital ichthyosiform erythroderma
CISI: Congenital Ichthyoses Severity Index
DC: Dendritic cell
DC-LAMP: Dendritic cell lysosomal-associated membrane protein
DEFB4: β-Defensin-B4
EI: Epidermolytic ichthyosis
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
LCN2: Lipocalin 2
LI: Lamellar ichthyosis
LOR: Loricrin
NS: Netherton syndrome
PAR2: Protease-activated receptor 2
PPL: Periplakin
TEWL: Transepidermal water loss
TSLP: Thymic stromal lymphopoietin

Despite elucidation of the genetic basis for the various forms of ichthyosis, their underlying molecular mechanisms are poorly understood, with our knowledge predominantly based on culture and animal models.[19-29] These model systems chiefly focus on abnormal barrier function and lipid homeostasis, with little attention paid to immune disturbances.[6,30,31] Human studies, largely limited to Netherton syndrome (NS) and the lamellar ichthyosis (LI) phenotype of autosomal recessive congenital ichthyosis (ARCI), have examined just a few cytokines.[32-38] Blood analyses found inconsistent T_H2 skewing[39] and increases in levels of proinflammatory cytokines (TNF-α, IL-1β, IL-2, and IL-18).[40-42] Skin studies showed increased expression of TNF-α and IL-1β in patients with ARCI-LI[35] and of protease-activated receptor 2 (PAR2),[32] thymic stromal lymphopoietin (TSLP), TNF-α, IL-8,[43] and the T_H2 cytokine IL-33 in patients with NS,[38] which are often coupled with increased expression of terminal differentiation products (ie, filaggrin [FLG], loricrin [LOR], and involucrin), and lipid impairement.[32,35,37,38] Studies of response to systemic treatments, including retinoids (n = 11), anti-TNF (n = 1), and oral corticosteroids combined with omalizumab (n = 1), in patients with ARCI-LI and those with NS, respectively,[33-35] have only assessed a few cytokines. Therapy-induced decreases in IL-1β, IL-8, TSLP, IL-5, and IL-17A levels were found in patients with NS, whereas IL-1α and TNF-α levels were decreased (nonsignificantly) in patients with ARCI-LI.

To elucidate the basis for the cutaneous inflammation seen in patients with ichthyosis and its correlation with clinical characteristics, we analyzed skin from 21 patients with the most prevalent orphan forms of severe ichthyosis: ARCI-LI, ARCI–congenital ichthyosiform erythroderma (CIE), epidermolytic ichthyosis (EI), and NS. All subtypes showed cutaneous skewing of T_H17 expression, which correlated with disease severity. This T_H17 profile most closely resembled that of psoriasis, in which IL-17 antagonism is highly effective in reversing the inflammation and epidermal pathology.[44-47] These data can lead to a new treatment paradigm targeting the T_H17/IL-23 pathway in patients with ichthyosis.

METHODS

Patients' characteristics

Twenty-one patients (aged 10-57 years) with ichthyosis and known mutations were enrolled (Tables I and II and see Table E1 and the Methods section in this article's Online Repository at www.jacionline.org). Written institutional review board–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 Numeric Rating Scale), photography, and TEWL measurement were captured. Few scoring instruments have been used for ichthyosis severity, and the only one tested for reliability is the Congenital Ichthyoses Severity Index (CISI). In addition to scoring erythema/redness and hyperkeratosis/scaling, CISI measures alopecia (not a feature in most patients) and does not score potential differences in body regions.[48] Given its limitations, we modified the CISI scale, eliminating alopecia and prorating intensity based on body region and extent to create a composite score similar to the Psoriasis Area and Severity Index.[49] This Ichthyosis Area and Severity Index (IASI) measures the severity of the erythema (Ichthyosis Area Severity Index–Erythema [IASI-E]) and scaling (Ichthyosis Area Severity Index–Scaling [IASI-S]), adding them together to a total IASI score (Tables I and II and see Table E2 and the Methods section in this article's Online Repository at www.jacionline.org).

Four-millimeter biopsy specimens were collected and assessed in parallel with tissue from healthy subjects, patients with atopic dermatitis (AD), and patients with psoriasis previously published by our group.[50-54] Genotyping for *FLG* mutations in the AD cohort was previously performed on 4 patients, and results were negative.[51] Four samples of healthy adolescents were included (see Table E3 in this article's Online Repository at www.jacionline.org) for comparison with the younger ichthyosis cohort. Patients' characteristics are presented in Tables I and II and Table E1 and E3.

Quantitative RT-PCR

RT-PCR was performed, as previously described.[55,56] Expression values were normalized to human acidic ribosomal protein (hARP).

Immunohistochemistry

Immunohistochemistry (IHC) was performed on frozen sections, as previously described.[57] Antibodies are shown in Table E4 and cell counts are shown in Table E5 in this article's Online Repository at www.jacionline.org.

Statistical analyses

Except for RT-PCR expression values, no other missing value imputations were performed. All available observations were included in analyses, which were performed by using the statistical language R (www.R-project.org). Differences in expression values (in $\log_2$ scale), cell counts, and clinical variables were assessed by using linear models, which were age adjusted to account for significant differences in age distributions.

Unsupervised hierarchical clustering of variables or samples/patients was performed by using the Pearson correlation coefficient as a distance metric with the McQuitty agglomeration algorithm. The results are represented as a heat map with a dendrogram and a tree or phylogram (using R package *ape*). The uncertainty of the hierarchical clustering analysis was assessed by using multiscale bootstrap resampling (extended statistics are shown in the Methods section in this article's Online Repository).

RESULTS

Demographics and clinical characteristics of patients with ichthyosis

Twenty-one patients aged 10 years or greater with ARCI-CIE (n = 6), ARCI-LI (n = 7), EI (n = 5), or NS (n = 3) and with

TABLE I. Patients' demographics and clinical severity scores: Characteristics of patients with different ichthyosis subtypes

Characteristic	Parameter	Patients with CIE (n = 6)	Patients with LI (n = 7)	Patients with EI (n = 5)	Patients with NS (n = 3)	*P* value
Age (y)	Mean ± SD	26.4 ± 14.7	30.4 ± 14.8	27.6 ± 19.2	18.2 ± 5.3	.718
	Median (age range)	23.2 (10.8-45)	28.0 (10.8-57)	17.9 (11.6-55)	19.2 (12.5-23)	
Sex	Female	3	6	3	1	.511
	Male	3	1	2	2	
Race	White	5	5	5	2	.695
	Black	0	1	0	1	
	Asian	1	0	0	0	
	Hispanic	0	1	0	0	
Disease severity scores	IASI score ± SD	24.5 ± 11.1	29.7 ± 8.0	33.6 ± 6.3	27.3 ± 12.3	.437
	IASI-E ± SD	13.9 ± 8.9	10.3 ± 6.4	15.1 ± 6.7	16.2 ± 11.0	.652
	IASI-S ± SD	10.6 ± 4.7	19.4 ± 5.5	18.5 ± 4.9	11.1 ± 1.3	.017
	TEWL ($g/m^2/h$) ± SD	22.1 ± 5.4	16.2 ± 4.5	15.2 ± 6.8	28.1 ± 0.0	.040
	5-D itch scale ± SD	11.2 ± 3.1	13.8 ± 5.8	10.2 ± 3.1	17.0 ± 7.1	.268

TABLE II. Patients' demographics and clinical severity scores: Comparison of patients with ichthyosis versus healthy control subjects and patients with AD or psoriasis

Characteristic	Parameter	Control subjects (n = 16)	Patients with ichthyosis (n = 21; LS = 21)	*P* value, control subjects vs patients with ichthyosis	Patients with AD (n = 16; LS = 16; NL = 16)	Patients with psoriasis (n = 10; LS = 10; NL = 10)	*P* value, all groups
Age (y)	Mean ± SD	38.7 ± 17.1	26.8 ± 14.6	.033	52.8 ± 13.1	51.3 ± 11.0	<.001
	Median (age range)	44.5 (10.6-57)	23.0 (10.8-57)		49.5 (33-73)	54.0 (30-64)	
Sex	Female	7	13	.444	8	4	.609
	Male	9	8		8	6	
Race	White	13	17	.269	16	10	.201
	Black	0	2		0	0	
	Asian	0	1		0	0	
	Hispanic	3	1		0	0	
Disease severity scores			**IASI**		**SCORAD**	**PASI**	
	Mean ± SD	NA	28.9 ± 9.0		56.6 ± 10.7	20.3 ± 15.4	
	Median (IQR)	NA	29.7 [21.2-36.0]		55.0 [51.5-63.0]	14.2 [12.1-21.2]	

IQR, Interquartile range; *LS*, lesional; *NA*, not applicable; *NL*, nonlesional; *PASI*, Psoriasis Area and Severity Index.

known genetic mutations were included (Tables I and II and see Table E1).[58,59] All patients with LI had mutations in *TGM1*, encoding transglutaminase, which enables stratum corneum cross-linking[60]; patients with CIE had a range of mutated genes (see Table E1), particularly encoding proteins of the hepoxilin pathway.[1,61,62] All patients with EI had *KRT10* mutations, and those with NS had mutations in *SPINK5*,[63,64] encoding a protease inhibitor. Fig E1 in this article's Online Repository at www.jacionline.org shows representative clinical pictures of ichthyosis subtypes. Normal skin from healthy subjects (≥10 years, n = 16; Tables I and II and see Table E3) and lesional and nonlesional skin from adults with 2 common moderate-to-severe skin disorders, AD (n = 16) and psoriasis (n = 10), were also included for appropriate comparisons with all polar cytokine pathways (Tables I and II).[50-54] Because of age differences between groups, with ichthyosis being the youngest cohort ($P < .001$), all analyses were age adjusted (Tables I and II and detailed statistics are provided in the Methods section in this article's Online Repository).

There were no significant differences in IASI or IASI-E scores among subtypes (see Fig E1, *G* and *H*). However, patients with ARCI-LI and those with EI had greater IASI-S scores in comparison with patients with ARCI-CIE ($P < .01$ for patients with ARCI-LI and $P < .05$ for patients with EI) and patients with NS ($P < .05$ for patients with ARCI-LI, see Fig E1, *I*).

Two pruritus scores were measured: the Itch Numeric Rating Scale and the 5-D itch scale.[65] Because the 2 itch scales were highly correlated (see Fig E2 in this article's Online Repository at www.jacionline.org), the 5-D itch scale was used for correlations. The 5-D itch scale score was significantly higher ($P < .05$) for patients with NS compared with those with EI and those with ARCI-CIE (see Fig E1, *J*). Mean TEWL, a measure of barrier function, was also significantly greater in patients with NS compared with those with EI and those with ARCI-LI and in patients with ARCI-CIE compared with those with the EI subtype ($P < .05$; Tables I and II and see Fig E1, *K*).

Increased hyperplasia and cellular infiltration characterize ichthyotic skin

Epidermal hyperplasia (as measured based on epidermal thickness and mRNA and protein expression of keratin 16

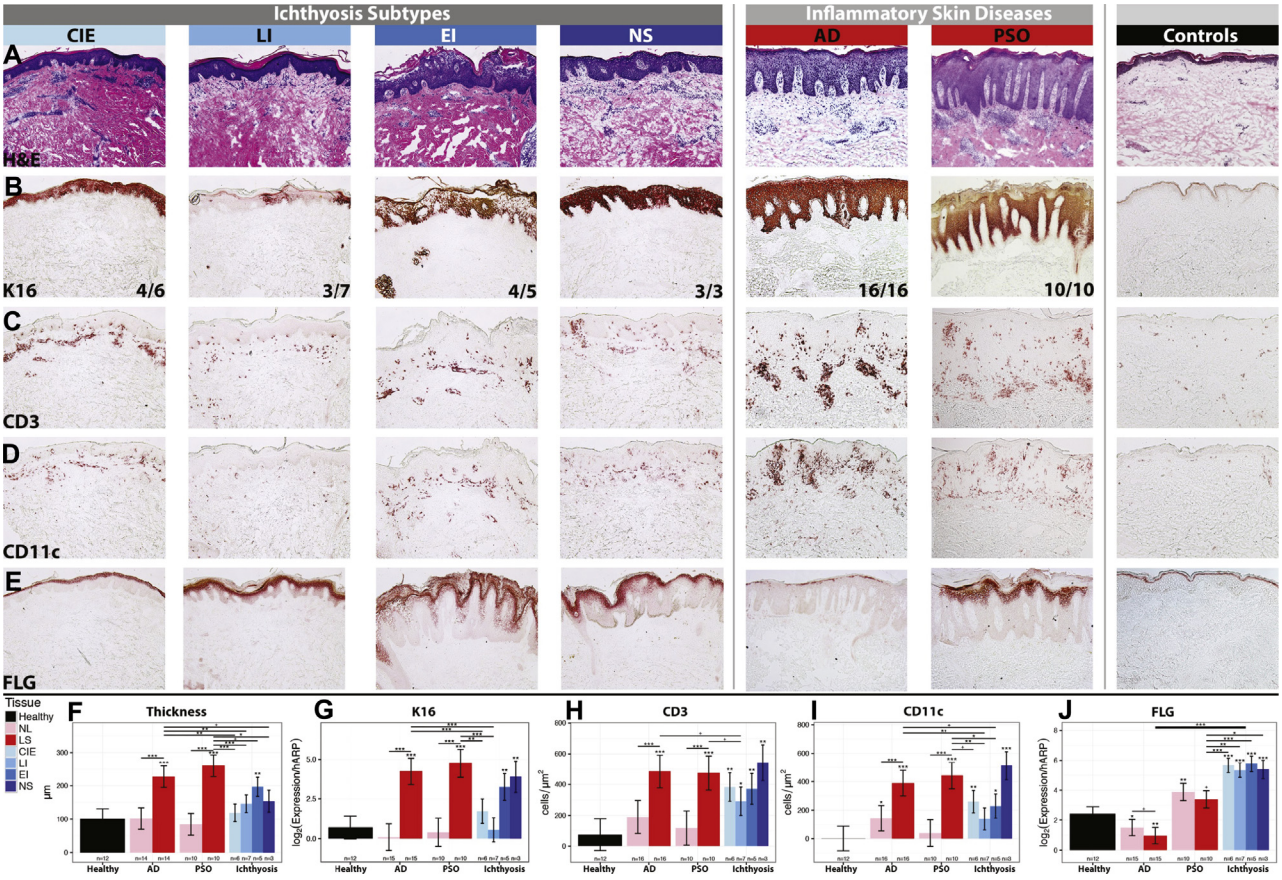

FIG 1. A-E, Representative staining in patients with ichthyoses, AD, or psoriasis *(PSO)* and control subjects by using hematoxylin and eosin (Fig 1, *A*), K16 with fractions of positive samples (Fig 1, *B*), $CD3^+$ T cells (Fig 1, *C*), $CD11c^+$ DCs (Fig 1, *D*), and FLG (Fig 1, *E*). **F-J,** Quantification of epidermal thickness (Fig 1, *F*), K16 mRNA (Fig 1, *G*), $CD3^+$ and $CD11c^+$ cells (Fig 1, *H* and *I*), and FLG mRNA (Fig 1, *J*). mRNA $\log_2$ values were adjusted to hARP. Results are presented as means ± SEMs. Control comparisons: $*P < .05$, $**P < .01$, and $***P < .001$. *LS*, Lesional; *NL*, nonlesional.

[K16], a marker of epidermal proliferation)[66,67] was seen in all patients with ichthyosis subtypes compared with control subjects. The greatest increases were observed in patients with NS (Fig 1, *A*). K16 staining in patients with ichthyosis was widespread and comparable with that of patients with lesional AD and psoriasis (16/16 and 10/10, respectively); the only exception was patients with LI, with only 3 of 7 $K16^+$ (Fig 1, *B*). The highest increases in epidermal thickness and K16 mRNA expression were seen in patients with EI and those with NS (Fig 1, *A*, *B*, *F*, and *G*). Significant increases in $CD3^+$ T-cell and $CD11c^+$ myeloid dendritic cell (DC) counts, dendritic cell lysosomal-associated membrane protein $(DC\text{-}LAMP)^+$ DC counts, and elastase-positive neutrophil counts characterized all ichthyosis subtypes compared with control subjects, with the greatest increases observed in patients with NS (Figs 1, *C* and *D*, and 2, *H* and *I*, and see Fig E3 in this article's Online Repository at www.jacionline.org). The infiltrates in patients with ichthyosis were comparable with those of highly inflammatory lesions from patients with AD and patients with psoriasis. In fact, patients with NS had similar increases in neutrophil counts (vs control skin) compared with those with psoriasis, which is considered a highly neutrophilic disease (see Fig E3).[54] Unlike patients with AD and those with psoriasis, there were no significant increases in $CD1a^+$ Langerhans cell counts (see Fig E3).

We also analyzed protein and mRNA expression of the keratinocyte differentiation markers (FLG, LOR, and periplakin [PPL]), which are largely downregulated in patients with AD.[11,68-70] 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 1, *E*). Similar to tissues from patients with psoriasis, most ichthyosis tissues, and particularly those from patients with 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 patients with ichthyosis than in those with psoriasis but largely suppressed in patients with AD compared with those in control subjects[69,71] and consistent with reduced FLG immunostaining (Fig 1, *J*, and see Fig E4 in this article's Online Repository at www.jacionline.org).

Ichthyotic skin shows T_H17-dominant inflammation

To evaluate expression of primary T_H1, T_H2, T_H9, T_H17, and T_H22 cytokines and some epidermal markers, which are often less than detection levels on gene arrays,[72] we performed quantitative real-time PCR. We observed large increases in the expression of general inflammatory (IL-2 and IL-15) and

some innate immune (IL-1β and IL-8) markers in skin from patients with ichthyosis compared with control skin (Figs 2 and 3, *A*, and see Fig E4). These increases were comparable and even higher than those in patients with AD and those with psoriasis. Interestingly, TNF-α expression was upregulated in patients with AD and those with psoriasis compared with that seen in control subjects but not in patients with ichthyosis, although higher levels were seen in patients with NS, as previously reported (Figs 2 and 3, *A*).[6,33,43] Expression of T_H1-related markers (IFN-γ, CXCL10, and CXCL9) was also increased in patients with ichthyosis compared with that in control subjects (Figs 2 and 3, *A*, and see Fig E4). The expression of T_H2 cytokines (IL-13, IL-31, IL-5, CCL17) was lower in patients with ichthyosis than in patients with AD and largely similar to that seen in control subjects (Figs 2 and 3, *A*, and see Fig E4). Expression of some T_H2 markers (CCL18, IL-10, and CCL22) was increased in patients with NS but to a much smaller degree than in patients with AD and comparable or even lower than in those with psoriasis (Figs 2 and 3, *A*, and see Fig E4). IL-9/T_H9 cytokine levels were not increased in patients with ichthyosis compared with those in control subjects (Fig 2).

Importantly, T_H17/IL-23 pathway genes were significantly induced, including those previously reported as synergistically or additively regulated by IL-17 and TNF-α (highlighted by green boxes in Figs 2 and 3, *A*, and see Fig E4).[73] Levels of IL-17A, p19 and p40 IL-23 subunits, IL-20, IL-23 receptor, and IL-17–induced chemokines (ie, human β-defensin 3) were increased, and upregulation of IL-17/TNF-α–synergistic/additive genes (IL-19, IL-17C, IL36G/IL1F9, PI3, CCL20, β-defensin-B4 [DEFB4], and S100A9) was particularly striking. Patients with NS had the highest induction of T_H17 pathway genes among ichthyosis subgroups, including the largest expression of IL-19, which is induced by both T_H17 and T_H2 cytokines and in turn amplifies the IL-17 effects in keratinocytes (Figs 2 and 3, *A*).[74-78] Although not directly induced by TNF-α, IL-19 is synergistically induced by IL-17 and TNF-α.[73] Many IL-17–related factors, including those displaying a synergistic/additive effect with TNF-α, showed comparable or even higher (IL-17C, CCL20, and IL36G) upregulation in patients with ichthyosis compared with that seen in patients with lesional psoriasis (Figs 2 and 3, *A*). Although IL-22/T_H22 levels were only mildly increased in patients with NS and those with ARCI-CIE, the S100As (S100A8/9/12), which are induced by both IL-17 and IL-22,[79] had significant increases in patients with ichthyosis versus control subjects. A cluster of IL-17–related and IL-17/TNF-α–synergistic/additive genes (IL17A/C, lipocalin 2 [LCN2], S100A8/12, IL36G, IL-20, and PI3) was upregulated in patients with ichthyosis to a similar extent as in those with psoriasis (and much higher than in patients with AD; highlighted green box in Fig 3, *A*). All RT-PCR values and comparisons are listed in Table E6 in this article's Online Repository at www.jacionline.org. Protein expression of key IL-17–induced antimicrobials and genes, CCL20, LCN2, and DEFB4, was also determined by using IHC. Wide epidermal expression was noted in patients with all ichthyosis subtypes and those with psoriasis, with minimal expression of DEFB4 in patients with AD (Fig 4).

To further evaluate how ichthyosis profiles relate phenotypically to psoriasis, we performed an unsupervised hierarchical clustering of ichthyosis, AD, psoriasis, and control skin by using expression profiles of all markers evaluated by using quantitative real-time PCR. Results are represented as a phylogenetic tree (Fig 3, *B*), showing tight clustering of ichthyosis and psoriasis lesions, whereas control subjects and patients with AD are much further apart. Of note, ichthyosis tissues did not subcluster by subtype. Similarly, we performed a comparison of gene expression changes between patients with *TGM1* (n = 8) and *ALOX12B* (n = 3) mutations and found no differences in expression other than IL-12RB2 ($P < .05$, data not shown).

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

To determine how clinical severity, as measured by using IASI and its subscores, 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 scores included IASI-E ($r = 0.74$), IL-17A ($r = 0.57$), IL-17–related markers (eg, PI3, $r = 0.61$), and the proliferation marker K16 ($r = 0.49$; $P < .03$; Fig 5, *A*, and see Table E7 in this article's Online Repository at www.jacionline.org). Highly significant correlations were found between IASI-E scores and IL-17A levels ($r = 0.74$) and expression of IL-17–related or IL-17/TNF–synergistic genes (CXCL1, PI3, IL36G, and S100As and IL-23p19, DEFB4, and LCN2; $P < .005$). Significant correlations were also noted between IASI-E scores and K16 levels and other immune (IL-1β) or cellular (DC-LAMP$^+$) markers (Fig 5, *B*, and see Table E7). IASI-S scores were significantly correlated only with IASI scores. The 5-D Itch scale showed few nonsignificant correlations. TEWL showed significant correlations with many IL-17–related markers (ie, IL-17A/IL-17-C, LCN2, and CXCL1) and TNF-α (Fig 5, *C*, and see Table E7).

To evaluate how different clinical scores (IASI, IASI-E, IASI-S, pruritus, and 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 by 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 6, *A*). A tight cluster was found between IASI-E scores and IL-17–induced or IL-17/TNF-α–synergistically modulated markers (eg, IL-17A, CXCL1, DEFB4, and PI3), supporting the link between IL-17 activation and ichthyosis erythema. In proximity to this cluster are 2 clusters of IL-17/IL-23/TNF-α–related genes (IL-22, IL-12/23p40 and IL-17C, IL-20; Fig 6, *A*). TEWL clustered with IL-22 and TNF-α and close to IL-17 markers, and the thickness measure clustered with IASI-S scores and close to terminal differentiation markers (LOR, LOR, and PPL), reflecting a possible link between barrier and immune measures. The 5-D itch scale closely clustered with T_H2 markers, including IL-13, IL-5, the itch cytokine IL-31,[80,81] and CCL26. Markers of T cells (CD3$^+$), DCs (CD11c$^+$ and DC-LAMP$^+$), and neutrophils clustered together and in proximity to a large cluster of IL-17/IL-23–related and other immune genes (Fig 6, *A*).

These data are also presented as a heat map showing positive (red) or negative (blue) correlations of all molecular and cellular measures and clinical measures in patients with ichthyosis (Fig 6, *B*), with color intensity reflecting the correlation's strength. A green box shows the associations of IASI-E scores with expression of IL-17A and other IL-17–related genes (that clustered together in the phylogenetic tree).

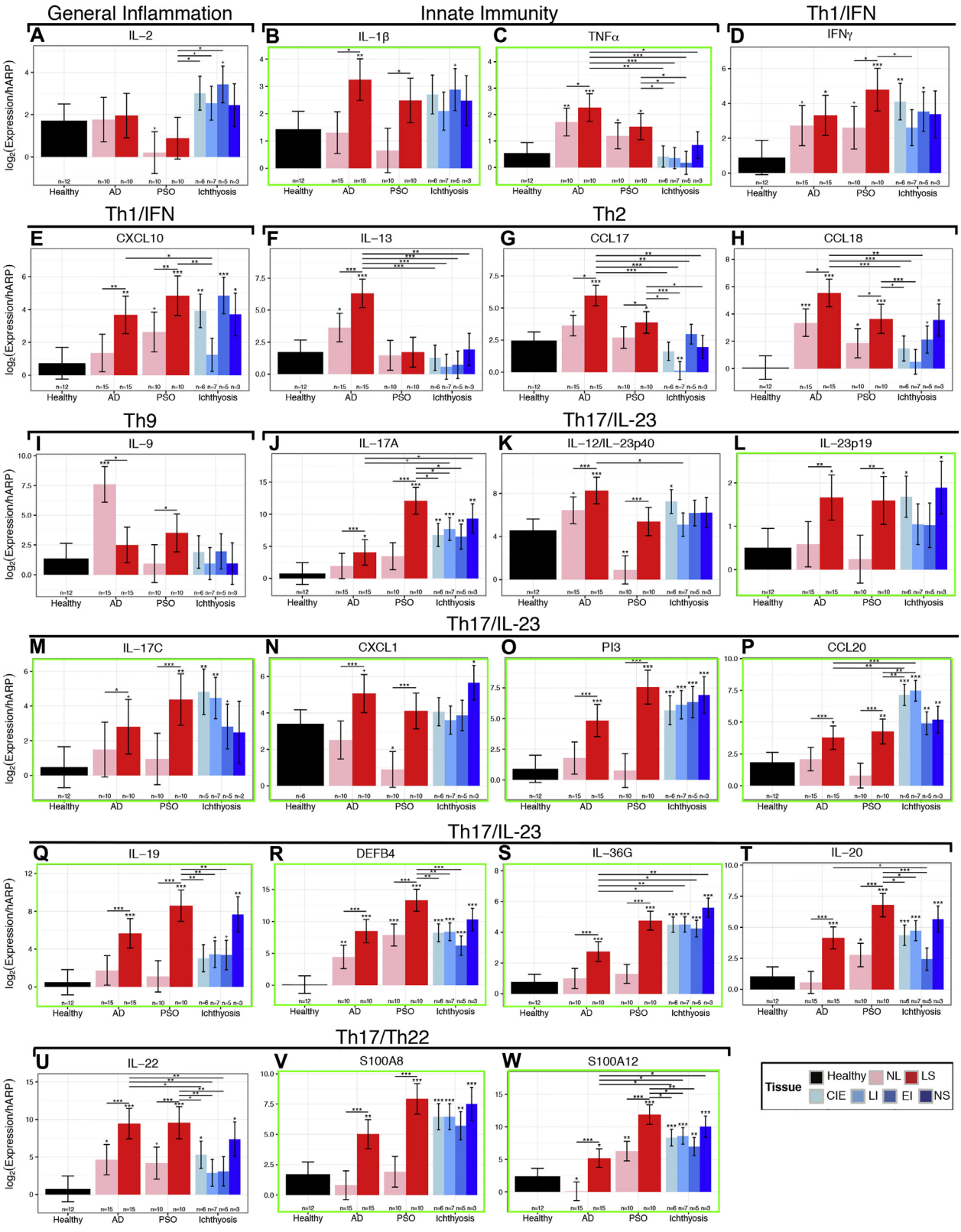

FIG 2. A-W, Comparison of immune markers in patients with ichthyosis subtypes, AD, or psoriasis *(PSO)* and control subjects by using RT-PCR. IL-17/TNF-α–synergistic/additive genes are highlighted in green. mRNA $\log_2$ values were adjusted to hARP expression levels. *Asterisks without bars* denote comparison with control subjects. *Asterisks above bars* denote *P* values, with comparators defined by the bar. Values are presented as least-square means ($\log_2$ expression/hARP) ± SEMs. $+P < .1$, $*P < .05$, $**P < .01$, and $***P < .001$.

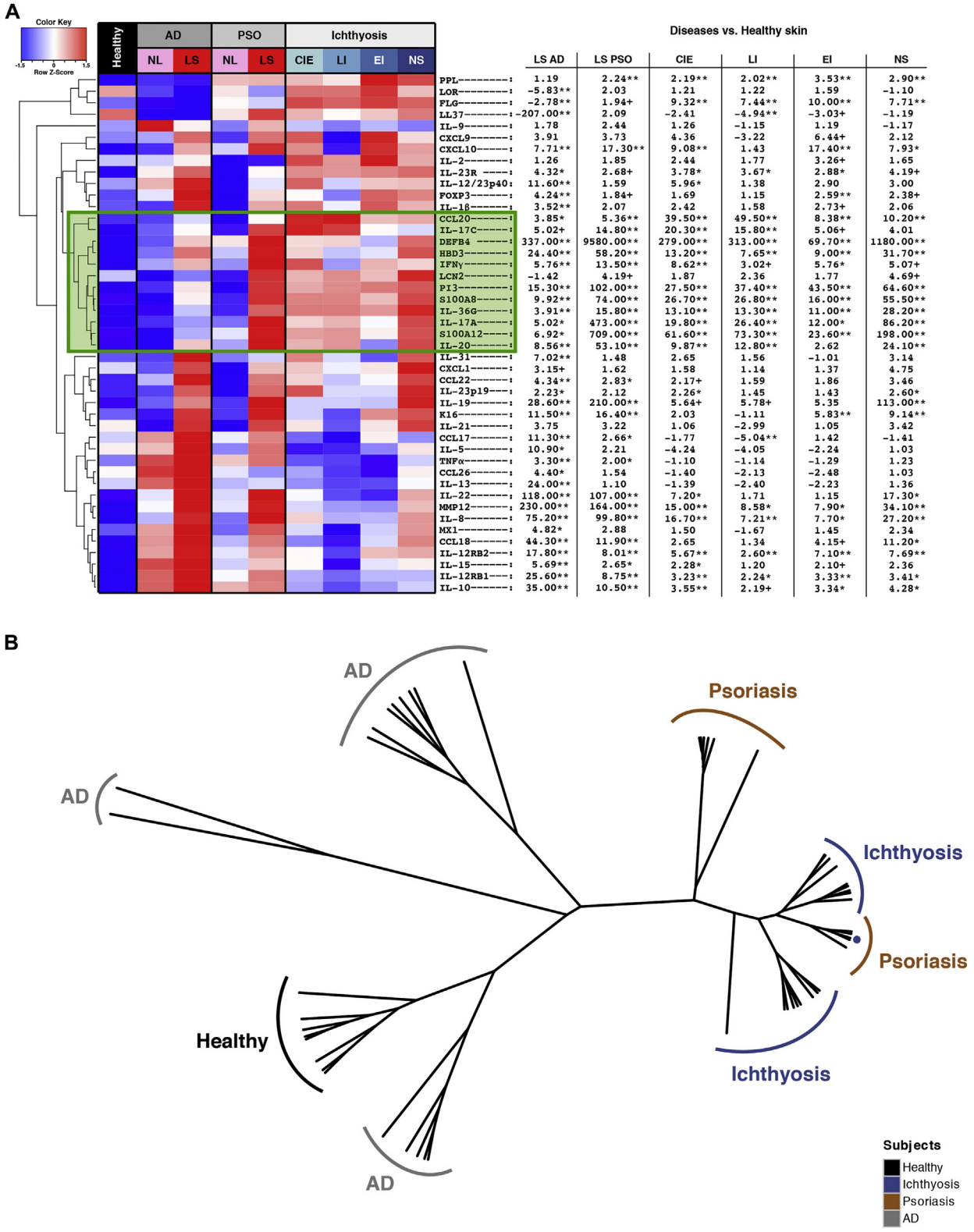

FIG 3. A, Unsupervised hierarchical clustering of mRNA expression in patients with AD, psoriasis, or ichthyosis and control subjects as a heat map with fold changes between diseased and healthy skin. *Green box,* Cluster of upregulated IL-17–related genes in patients with ichthyosis and those with psoriasis. $+P < .1$, $*P < .05$, and $**P < .01$. *Red,* Upregulation; *blue,* downregulation. **B,** Unsupervised clustering of samples (phylogenetic tree) based on expression profiles of 45 immune/barrier markers. Distance, Pearson correlation; agglomeration, average. *LS,* Lesional.

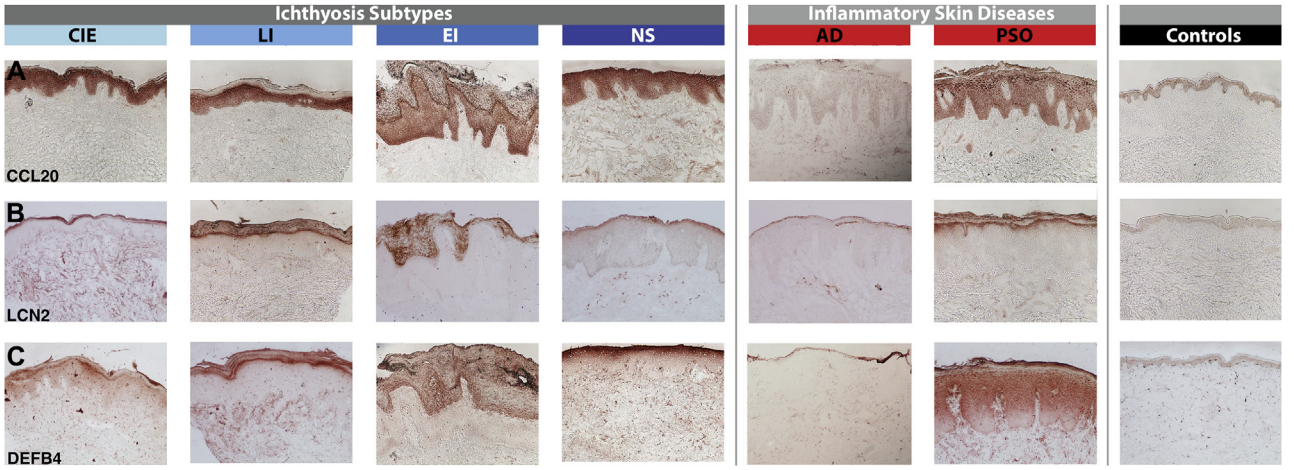

FIG 4. Representative IHC staining of IL-17 induced antimicrobials in patients with ichthyoses, AD, or psoriasis *(PSO)* and control subjects by using CCL20 **(A)**, LCN2 **(B)**, and DEFB4 **(C)**, showing strong protein expression in the skin of patients with ichthyoses similar to what is seen in the skin of patients with psoriasis.

Associations with scaling/thickness, TEWL, and 5-D itch scale score are highlighted by pink, brown, and gray boxes, respectively. Individual correlations with clinical scores are shown in Table E7.

DISCUSSION

The ichthyoses are primarily rare, life-altering genetic disorders characterized by scaling, epidermal thickening, and erythema.[1,5] Available systemic treatments (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.[13,16]

Few studies have evaluated the role of immune dysregulation in patients with ichthyosis,[33-35] with the underlying molecular basis predominantly based on limited data from *in vitro* and animal models.[6,19-31] These models observed proinflammatory signals, with increases in cytokine (IL-1 and TNF-α) and chemokine (S100As, CXCL1, TSLP, and PAR2) levels and parallel epidermal hyperplasia (increases in K16 and K6B) and abnormalities in differentiation (LOR and FLG) and lipid genes.[22,24,30,31,82] Mouse models of NS showed diverse cytokine activation with increases in levels of innate, T_H2, T_H17, and T_H22 cytokines (IL-1β, TNF-α, IL-4, Il-13, IL-17, and IL-22) and corresponding chemokines (TSLP, CCL17, CXCL1, CCL20, and S100A8/9).[20] 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.[22,31]

As noted previously, the few investigations with human ichthyotic skin focused primarily on barrier alterations (hyperplasia, premature expression of terminal differentiation products, and lipid defects).[32-38] In the few studies of blood or cultured ichthyotic keratinocytes, levels of selected polar cytokines were increased[39-42] and reduced by immune modulators.[33-35] IL-17 levels are also increased in a mouse model of AD with FLG deficiency and in patients with AD with *FLG* mutations.[83]

This is the first extensive molecular profiling of ichthyosis subtypes. Because ichthyoses share clinical and histologic characteristics with 2 common skin disorders, AD and psoriasis (ie, 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 nonlesional skin from patients with moderate-to-severe AD and psoriasis, as well as skin from healthy volunteers. This approach allowed determination of cytokine pathway upregulation in patients with ichthyosis and comparison with AD (primarily T_H2 driven) and psoriasis (primarily T_H17/IL-23 driven). In these diseases inflammatory responses play an important role in disease progression, including in the epidermal component.[45,47,51-53,84-88] Advances in understanding pathogenesis have translated into rapid development of cytokine-targeted therapeutics, which reverse the clinical inflammation but also the epidermal disease phenotype.[84,86-96] Pathogenesis-based therapies are available for psoriasis based on its T_H17/IL-23–centered activation. TNF-α has been functionally linked to the T_H17/IL-23 pathway, and TNF antagonists are highly effective for psoriasis.[73,97] Furthermore, psoriasis treatment with etanercept, a TNF inhibitor, suppresses genes that are synergistically induced by IL-17 and TNF to a greater extent than TNF-α–regulated genes alone.[73]

Our data show that all ichthyoses share impressive T_H17/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, and CCL20) despite lower TNF-α modulation. IL-36G has been reported to amplify TNF-α and IL-17 pathways in patients with psoriasis and to accurately differentiate psoriasis from AD lesions.[98] The induction of genes modulated by IL-17 alone or IL-17 and TNF-α together was largely comparable with that seen in patients with psoriasis,[99,100] perhaps leading to tight clustering of ichthyoses and psoriasis samples. This was unexpected, given the pruritic nature of many patients with ichthyoses (especially those with ARCI-CIE or NS) and their clinical resemblance to AD.[101] However, mRNAs of T_H2 markers (eg, IL-5, IL-13, IL-31, CCL17, and CCL26) in all ichthyosis subsets were surprisingly low. The only exception was in patients with NS, which is traditionally linked to atopy, in whom some T_H2 markers (CCL18 and CCL22) were increased. The concomitant increases

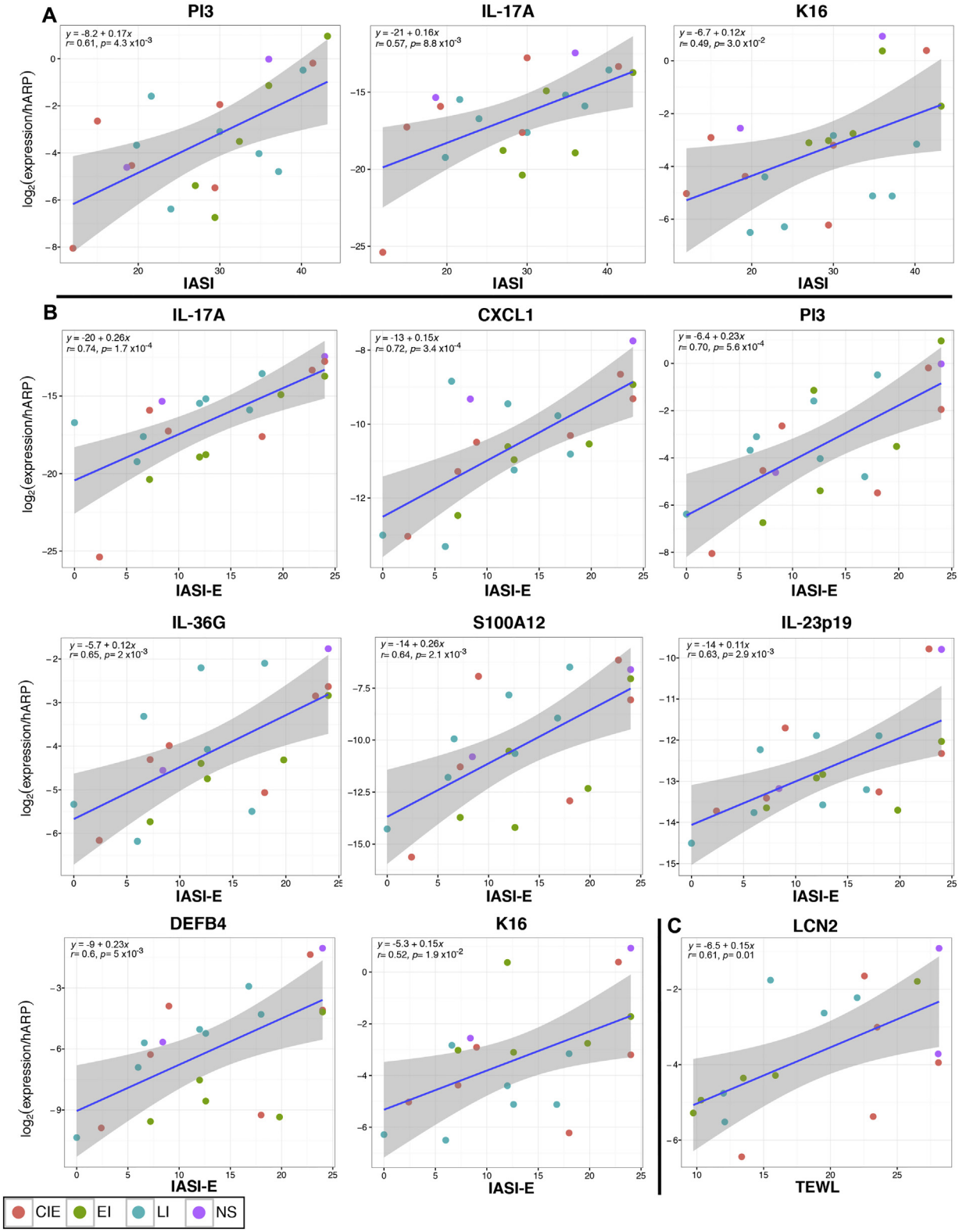

FIG 5. Pearson correlation plots of mRNA gene expression that correlated highest with overall clinical severity scores (IASI; **A**), erythema severity subscores (IASI-E; **B**), and TEWL **(C)** in patients with ichthyosis subtypes. *r,* Pearson correlation coefficient with associated *P* value; *y,* equation for linear regression *(blue line)* with its CI (smoothed CI in *gray*).

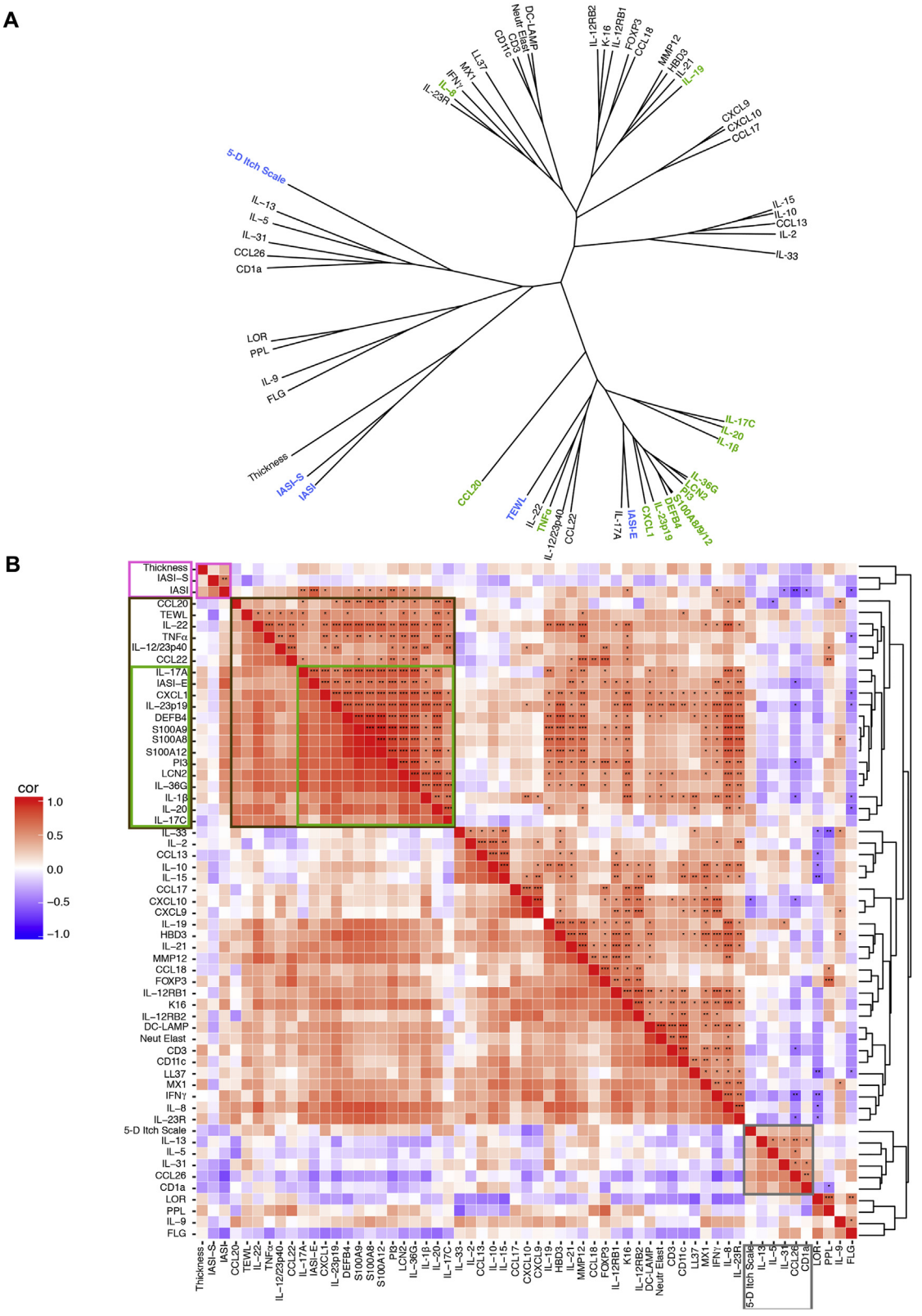

FIG 6. Correlation matrix of all ichthyosis measurements. **A,** Unsupervised hierarchical clustering of clinical scores *(blue)* with barrier/immune markers *(black)*, including IL-17–synergistic/additive genes *(green)*. Distance, Pearson correlation; algorithm, McQuitty agglomeration. **B,** Correlation heat map. *Pink box*, Correlations with IASI score. *Brown box*, Most significant cluster of IL-17– synergistic/additive genes, with the *green box* highlighting the IASI-E subcluster. *Gray box*, Pruritus correlations. *red*, Positive correlations; *blue*, negative correlations. $*P < .05$, $**P < .01$, and $***P < .001$.

in T_H17- and T_H2-related markers in patients with NS might also contribute to the large increases in IL-19 levels in this subtype, given that T_H17 but also T_H2 cytokines can induce IL-19.[74-78] IL-19 induces epidermal hyperplasia and S100As,[74-78] which were highest in patients with NS. Interestingly, although mouse NS models demonstrate increased T_H2/T_H17 responses,[6,20,21] T_H2 inhibition through PAR2/TSLP suppression did not improve cutaneous inflammation.[30] Expression of T_H1 markers varied in patients with different ichthyoses but was mostly lower than in patients with AD and those with psoriasis. Increases in innate markers (IL-1β and IL-8) were also seen in patients with ichthyoses and largely comparable with those in patients with AD and those with psoriasis.

Importantly, IASI scores, particularly the erythema subscore IASI-E (reflecting clinical severity), were highly correlated with IL-17A and IL-17/TNF–regulated genes (CXCL1 and IL-36G). Significant correlations were also found between IASI and IASI-E scores and epidermal hyperplasia, as measured by K16.[66,102] Although in patients with AD and those with psoriasis epidermal hyperplasia is linked to IL-22, IL-22 activation was far lower in patients with ichthyosis than in those with AD or psoriasis. Other hyperplasia-inducing IL-20 family cytokines (ie, IL-19) might contribute to increased the epidermal thickness in patients with ichthyosis.[103-106]

The ichthyoses are recognized as resulting in significant epidermal abnormalities,[67,71,107,108] including epidermal hyperplasia, and lipid and differentiation abnormalities leading to barrier impairment, which is reflected by increased TEWL.[1,3] Hyperplasia and differentiation abnormalities, particularly in patients with ARCI and NS subtypes, are similar to those seen in patients with psoriasis, with hyperplastic epidermis and largely increased expression of differentiation proteins (LOR, FLG, and PPL) in the upper epidermis.[32,43,109] We found higher expression of these markers in patients with ichthyosis and psoriasis but much reduced expression in patients with AD. The significant correlations between TEWL with IL-17A and IL-17/TNF–regulated genes (IL-17C, CXCL1, LCN2, and IL-36G) might link the immune activation and functional barrier abnormalities in patients with ichthyosis.

Finally, similar to those with psoriasis, patients with ichthyosis are able to mount significant IL-17–induced antimicrobial peptide (AMP) responses, as shown by high mRNA expression of LL37, DEFB4, human β-defensin 3, and CCL20. Our IHC studies of DEFB4, LCN2, and CCL20 show increased protein expression of IL-17–related proteins/AMPs. AMPs were recently shown to upregulate tight junctions and keratinocyte differentiation,[110,111] providing an explanation beyond antimicrobial function for increases in these products in patients with ichthyosis and those with psoriasis versus patients with AD. A critical consideration in interpreting these results is whether the increase in T_H17/IL-23 pathway expression is merely a compensatory mechanism in an effort to reduce the risk of infection versus a driver of inflammation. Based on case reports and our experience, *Staphylococcus aureus*, dermatophyte, and candidal infections occur not infrequently in patients with ichthyosis, although *S aureus* infections are less common than in those with moderate-to-severe AD.[99,112-116] Even with an anti–IL-17 drug (secukinumab) for psoriasis, only about 1% of patients have mild-to-moderate mucocutaneous infections, predominantly candidal.[117,118] The reactive role of T_H17/IL-23 skewing to the barrier defects versus a primary pathogenic role in patients with ichthyosis can only be clarified through future studies with targeted antagonists, which include careful monitoring for mucocutaneous infections.

Limitations of our data include the small sample size, reflecting the rarity of ichthyoses, and the use of a new, nonvalidated severity score (chosen because no acceptable validated score was available). 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.

Although 25% of control subjects were children 10 years or older, the age difference between patients with ichthyosis and healthy subjects was statistically significant. Our AD and psoriasis samples were obtained from subjects 18 years or older, although 8 of 21 patients with ichthyosis were less than 18 years old. Although the adolescent skin phenotype in patients with AD and those with psoriasis is commonly considered close to that in adults, there are no studies comparing the two. Thus, for proper comparisons, all analyses were age adjusted, and a sensitivity analysis including only patients with 18 years old and older suggested similar findings (see Table E8 in this article's Online Repository at www.jacionline.org). Furthermore, given that IL-17 expression increases with age in healthy skin,[119,120] our results might actually underestimate the increased T_H17 activation in patients with ichthyosis. Future studies should address the effect of age on the observed differences.

Our data link the T_H17/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 patients with 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 patients with ichthyosis. One patient with NS demonstrated clinical improvement and reduction in IL-17 levels after administration of infliximab (anti–TNF-α used to treat psoriasis).[33,121,122] IL-17/IL-23–targeting strategies[94,123-125] have been shown to be more effective than TNF-α inhibitors in patients with psoriasis, dramatically improving Psoriasis Area and Severity Index scores.[92,126] Specific IL-17/IL-23 targeting will elucidate a functional role of IL-17 in patients with ichthyoses and might establish a novel treatment paradigm for ichthyoses.

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Clinical implications: The link between increased expression of T_H17 pathway cytokines and clinical disease severity raises the possibility of a new therapeutic paradigm of targeted IL-17/IL-23 intervention for patients with ichthyosis.

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