

Early-onset pediatric atopic dermatitis is characterized by T_H2/T_H17/T_H22-centered inflammation and lipid alterations

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Background: Although atopic dermatitis (AD) often starts in early childhood, detailed tissue profiling of early-onset AD in children is lacking, hindering therapeutic development for this patient population with a particularly high unmet need for better treatments.

Objective: We sought to globally profile the skin of infants with AD compared with that of adults with AD and healthy control subjects.

Methods: We performed microarray, RT-PCR, and fluorescence microscopy studies in infants and young children (<5 years old) with early-onset AD (<6 months disease duration) compared with age-matched control subjects and adults with longstanding AD.

Results: Transcriptomic analyses revealed profound differences between pediatric patients with early-onset versus adult patients

with longstanding AD in not only lesional but also nonlesional tissues. Although both patient populations harbored T_H2-centered inflammation, pediatric AD also showed significant T_H17/T_H22 skewing but lacked the T_H1 upregulation that characterizes adult AD. Pediatric AD exhibited relatively normal expression of epidermal differentiation and cornification products, which is downregulated in adults with AD. Defects in the lipid barrier (eg, ELOVL fatty acid elongase 3 [ELOVL3] and diacylglycerol o-acyltransferase 2 [DGAT2]) and tight junction regulation (eg, claudins 8 and 23) were evident in both groups. However, some lipid-associated mediators (eg, fatty acyl-CoA reductase 2 and fatty acid 2-hydroxylase) showed preferential downregulation in pediatric AD, and lipid barrier genes (FA2H and DGAT2) showed inverse correlations with transepidermal water loss, a functional measure of the epidermal barrier.

Conclusions: Skin samples from children and adult patients with AD share lipid metabolism and tight junction alterations, but epidermal differentiation complex defects are only present in adult AD, potentially resulting from chronic immune aberration that is not yet present in early-onset disease. (*J Allergy Clin Immunol* 2018;■■■:■■■-■■■.)

Key words: Atopic dermatitis, pediatric, infant, skin barrier, skin inflammation

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Atopic dermatitis (AD) is the most common chronic inflammatory skin disease, affecting up to 20% of children and 7% to 10% of adults.¹⁻³ A strong immune infiltrate and impaired skin barrier are thought to characterize the disease,⁴ but their initial sequence and exact interplay are still not fully understood.⁵ AD usually begins before age 5 years, with approximately 60% of patients experiencing disease onset within the first year of life.² However, skin data are largely limited to samples from adolescents and adults with years to decades of disease activity,⁶⁻⁹ raising the possibility of considerable differences compared with early AD in infants.^{10,11}

In a recent study our group assessed selected inflammatory and barrier markers in pediatric patients less than 5 years of age within 6 months of disease onset.¹⁰ Using immunohistochemistry and quantitative RT-PCR, we found T_H2 activation, as in adult AD skin. However, we also noted strong upregulation of T_H17/IL-23 responses and antimicrobial peptide (AMP) mRNA expression in young children¹⁰ similar to levels seen in psoriasis and in contrast to the relatively reduced AMP expression in acute and chronic adult AD.¹² In addition, filaggrin (FLG), a skin barrier protein that has been implicated as a central player for

Abbreviations used

AD:	Atopic dermatitis
CLDN:	Claudin
DEG:	Differentially expressed gene
EDC:	Epidermal differentiation complex
ELOVL:	Elongation of very long chain fatty acids protein 3
EREG:	Epiregulin
FLG:	Filaggrin
KYNU:	Kynureninase
LCE:	Late cornified envelope
STAT:	Signal transducer and activator of transcription
TEWL:	Transepidermal water loss

AD development because of cytokine-mediated downregulation (in addition to loss-of-function mutation in a minority of AD patients)^{13,14} was still present at normal levels in pediatric early-onset AD. Despite intact FLG expression, a barrier defect was present in early-onset AD, as reflected by high levels of transepidermal water loss (TEWL),¹⁰ a discrepancy that remained unexplained.

Thus a global profiling of AD initiation in both lesional and nonlesional skin compared with skin tissue from both age-matched control children and adults with chronic AD of similar severity is lacking. This global molecular profiling was performed to gain better insight into pathogenic mechanisms that are potentially operative during initial AD. We found that early disease initiation has substantial differences compared with adult AD, with strong T_H2- and T_H17/T_H22-centered inflammation but lacking T_H1 activation and terminal differentiation abnormalities, as seen in adults.

METHODS**Patients' characteristics**

Nineteen children 3 months to 5 years of age (mean age, 1.3 years) with AD onset within the previous 6 months and moderate-to-severe disease (SCORAD score: mean, 57.8; range, 33-84)¹⁰ were included in analyses (Table I).¹⁵ Parents signed institutional review board–approved consent forms before study inclusion. No heterozygous *FLG* mutations (R501X and 2282del4 allele) were found in any pediatric patient with AD. The *FLG* mutation status for 2282del4 was unavailable for 1 patient because of an inadequate sample. Systemic immunosuppressants within the past 4 weeks, topical steroids or immunomodulators within 1 week, or moisturizers within 12 hours before evaluation were restricted. Patients with active skin infections were excluded. TEWL was measured at biopsy sites by using a Tewameter (Courage and Khazaka GmbH, Cologne, Germany; Table I). Lesional and nonlesional 4-mm biopsy specimens from affected popliteal and unaffected buttock skin, respectively, were obtained. All biopsy specimens were from chronic lesions present for more than 72 hours.¹¹ Pediatric control skin was collected during routine surgical procedures from a group of 18 healthy subjects matched for age, sex, and ethnicity without personal/familial atopy. Lesional and nonlesional tissues from adults with AD (n = 20; age range, 18-73 years) with similar disease severity (SCORAD score: mean, 62.4; range, 44-97) and matched control skin (n = 11; age range, 38-57 years) from previously reported cohorts¹⁶⁻¹⁸ were included as comparators (Table I).

Visualization of skin lipid deposition

Fresh cryostat sections were stained with Nile red solution (500 mg/mL) in acetone, and images were acquired by using appropriate filters of the Zeiss Axioplan 2 (LSM 510; Carl Zeiss Microimaging, Thornwood, NY), as previously reported.¹⁹

Quantitative RT-PCR and microarray analysis

RNA was extracted for RT-PCR with EZ-PCR Core Reagents (Life Technologies, Grand Island, NY), as previously described.²⁰ Expression levels were normalized to human acidic ribosomal protein. HGU133Plus2.0 microarrays (GSE#107361; Affymetrix, Santa Clara, Calif) were used, as previously described.^{11,20,21}

Statistical analyses

Statistical analyses were performed with R software (www.R-project.org), with respective packages available through Bioconductor (www.bioconductor.org). Standard quality control metrics and the R package microarray QualityControl were used for microarrays, as previously described.²⁰ Images were scrutinized for artifacts by using Harshlight.²² Expression measures were obtained by using the GCRMA algorithm.²³ Batch effects were adjusted with Combat.^{20,24} Unsupervised hierarchical clustering with Euclidean distance and McQuitty agglomeration were applied for heat maps.²³ Probe sets with expression values of greater than 3 were kept for analyses. Data from microarray experiments were log₂ transformed before analyses with a linear mixed-effects model. For AD samples, age category and tissue type (lesional, nonlesional, or control tissue) were used as fixed factors, with a random effect for sex and race/ethnicity. Fold changes were estimated, and hypothesis testing was conducted by using contrasts in the R limma package linear model framework.²⁰ The Benjamini-Hochberg procedure was used to adjust *P* values from paired *t* tests for multiple hypotheses. For RT-PCR, human acidic ribosomal protein–normalized expression levels were log₂-transformed before analyses. For each marker, mixed-effect models were fit with age category and tissue type as fixed factors and a random effect for sex and race/ethnicity.

RESULTS**Study patients**

Nineteen children less than 5 years old with early-onset AD (<6 months disease duration) and 18 age-matched pediatric control subjects, as well as a group of 20 adults with AD with comparable disease severity and 11 age-matched adult control subjects, were studied.

The pediatric AD skin phenotype shows substantial differences from longstanding disease in adults

A principal component analysis adjusted for batch effects showed distinct grouping of nonlesional, lesional, and healthy skin that differed from that in adult subjects (Fig 1, A). Using criteria of fold change of greater than 2 and a false discovery rate of less than 0.05, we assessed differentially expressed genes (DEGs) for lesional versus nonlesional AD (Fig 1, B) and lesional AD versus healthy control tissues (Fig 1, C). The pediatric lesional versus nonlesional comparison included 788 DEGs (424 upregulated and 364 downregulated; Fig 1, B) and 3078 DEGs in lesional AD versus healthy control tissues (1168 upregulated and 1910 downregulated; Fig 1, C). Only 22.6% (lesional vs nonlesional tissues) and 30.3% (lesional vs healthy control tissues) of DEGs in pediatric patients with AD were shared with the adult AD profile. Although key T_H2 and T_H22 genes implicated in AD pathogenesis^{4,25} were among the common AD profile (Fig 1, B and C, and see Table E1 in this article's Online Repository at www.jacionline.org) across adults and children (ie, CCL17/CCL18/CCL22/IL-4 receptor among T_H2 and S100A7/S100A8/S100A9/S100A12 among T_H22 genes), other genes associated with the adult AD profile,^{13,18,26} including *IFNGR1* (T_H1), *CCL20* (T_H17), and *FLG* (barrier), showed different regulation in children and adults (see Table E1).

TABLE I. Baseline characteristics

	Pediatric patients with AD (n = 19)	Pediatric control subjects (n = 18)	P value	Adult patients with AD (n = 20)	Adult control subjects (n = 11)	P value
Age (y), mean (SD)	1.3 (1.2)	1.2 (0.8)	.721	47.6 (14.5)	49.2 (7.1)	.745
Male sex, no. (%)	11 (57.9)	8 (44.4)	.517	12 (60.0)	10 (90.9)	.106
SCORAD score, mean (SD)	57.8 (12.8)	NA	—	62.4 (13.0)	NA	—
TEWL, lesional (g/h/m ²), mean (SD)*	32.1 (14.0)	NA	—	NA	NA	—
TEWL, nonlesional (g/h/m ²), mean, (SD)*	16.1 (8.0)	NA	—	NA	NA	—
Ethnicity (%)			.1140			.6468
Asian/Pacific Islander	2 (10.5)	2 (11.1)		1 (5)	0 (0)	
Black	5 (26.3)	1 (5.6)		1 (5)	1 (9.1)	
Hispanic	4 (21.1)	1 (5.6)		0 (0)	1 (9.1)	
White	8 (42.1)	14 (77.7)		18 (90)	9 (81.8)	

NA, Not available.

*Normal value (ie, mean TEWL in healthy children at month 2 has been reported to be 10.97 ± 7.98 g/m²/h and at month 6 has been reported to be 10.71 ± 7.1 g/m²/h by Kelleher et al).¹⁵

Immune gene expression in patients with early pediatric versus those with adult AD

Given that emerging AD treatments are designed to target specific inflammatory mediators, it is imperative to understand how immune circuits in children differ from those in adults. As a result, we compared the immune phenotype of lesional and nonlesional skin in children versus adults with AD by using a predefined immune gene subset,^{16,20,27} as displayed in a heat map sorted by using hierarchical clustering (Fig 2). Healthy pediatric and adult control subjects largely showed similar mRNA expression levels of immune markers, except for the innate cytokines IL-6, IL-1 β , and CXCL8/IL-8 and the T_H17-associated CCL20, peptidase inhibitor 3 (PI3)/elafin, IL-23A/IL-23p19, CXCL2, and signal transducer and activator of transcription (STAT) 3, levels of which were increased in children (Fig 2). The IFN- γ -associated cytokine IL-18, the IgE receptor subunit Fc ϵ RIA, and the T_H2-associated cytokine IL-33 were among the significantly decreased mediators in pediatric versus adult control skin (Fig 2). Pediatric patients already showed significant upregulation of inflammatory markers in their nonlesional skin (Fig 2). Impressive increases in nonlesional pediatric AD skin (compared with adults) were seen in T_H17/T_H22 products, including S100A7, S100A8, S100A9, and S100A12, as well as the T_H17- and T_H2-associated cytokine IL-19.^{28,29} Nonlesional pediatric AD lacked upregulation of several IFN- γ markers, including *STAT1*, *IFNGR1*, and interferon regulatory factor 1 (*IRF1*) and some T_H2-polarizing chemokines (CCL17, CCL18, and CCL26) in contrast to nonlesional adult AD. The trend of stronger upregulation of T_H17/T_H22 markers in nonlesional pediatric AD was also seen in lesional pediatric AD compared with adult AD (S100A7, S100A8, S100A9, S100A12, CXCL1, IL-22, and IL-26), as visualized in a cluster marked by a yellow box (Fig 2). In fact, numerous T_H17/T_H22-associated mediators (lipocalin 2 [LCN2], CCL20, IL23A/IL-23p19, IL-36A, IL-36B, IL-36G, and IL-36RN) and IL-19 were upregulated solely in pediatric lesions (Fig 2, green box). Conversely, a cluster containing several T_H1-associated mediators (CXCL9, CXCL10, STAT1, IFNGR1, and IL12RB2) showed stronger upregulation in adult AD lesions (Fig 2, blue box). Upregulation of general inflammation/T-cell activation markers (inducible T cell costimulator [ICOS] and matrix metalloproteinase 12 [MMP12]) and T_H2 markers characterized

both pediatric and adult lesions, including the T_H2-polarizing chemokines³⁰ CCL17, CCL18, CCL22, and the IL-4 receptor IL-4R (Fig 2, A, red box).

Pediatric AD shows upregulation of IL-17- and TNF- α -regulated genes

Because of the strong T_H17 skewing in pediatric AD, which is classically found in adult psoriasis,³¹ we evaluated AD expression levels of a subset of genes previously shown to be synergistically and/or additively regulated *in vitro* by IL-17 and TNF- α ,³² mimicking the psoriasis inflammatory milieu.³¹ As shown in Fig 3, the majority of IL-17/TNF- α -regulated genes were significantly upregulated in lesional pediatric compared with adult AD, which showed weaker-to-absent (ie, vanin3 [VNN3], kynureninase [KYN], S100A proteins, IL-19, peptidase inhibitor 3 [PI3]/elafin, and IL-24 [yellow box]) or absent-to-negative (ie, LCN2, CCL20, and TGF- α [green box]) regulation. Only a small cluster was upregulated exclusively in adult AD (within the blue box). To further confirm this trend, we performed quantitative RT-PCR on genes previously described as being highly upregulated in psoriasis but not in adult AD tissues, including TNF- α ,³³ CCL7,³⁴ KYN,³⁵ IL-36B,³⁶ IL-36G,³⁷ and epiregulin (EREG).³⁸ Indeed, these “psoriasis hallmark” markers showed significantly higher expression in early-onset pediatric AD compared with adult AD (Fig 4, A-F). Because of these differences, we assessed our pediatric tissues using a molecular disease classifier that had been validated for differentiating adult AD and psoriasis (Fig 4, G and H).⁷ This classifier evaluates levels of inducible nitric oxide synthase (ie, nitric oxide synthase 2 [NOS2]) and CCL27, with increased NOS2 levels characterizing psoriasis and normal or increased CCL27 levels associated with AD.^{7,39} Our pediatric samples clearly classified as AD, similar to their adult counterparts (Fig 4, G and H), confirming the clinical AD diagnosis despite the marked differences in the immune phenotype of children compared with adults with AD.

Barrier genes

In addition to aberrant immune activation, epidermal barrier impairment is thought to be a central pathogenic mechanism in

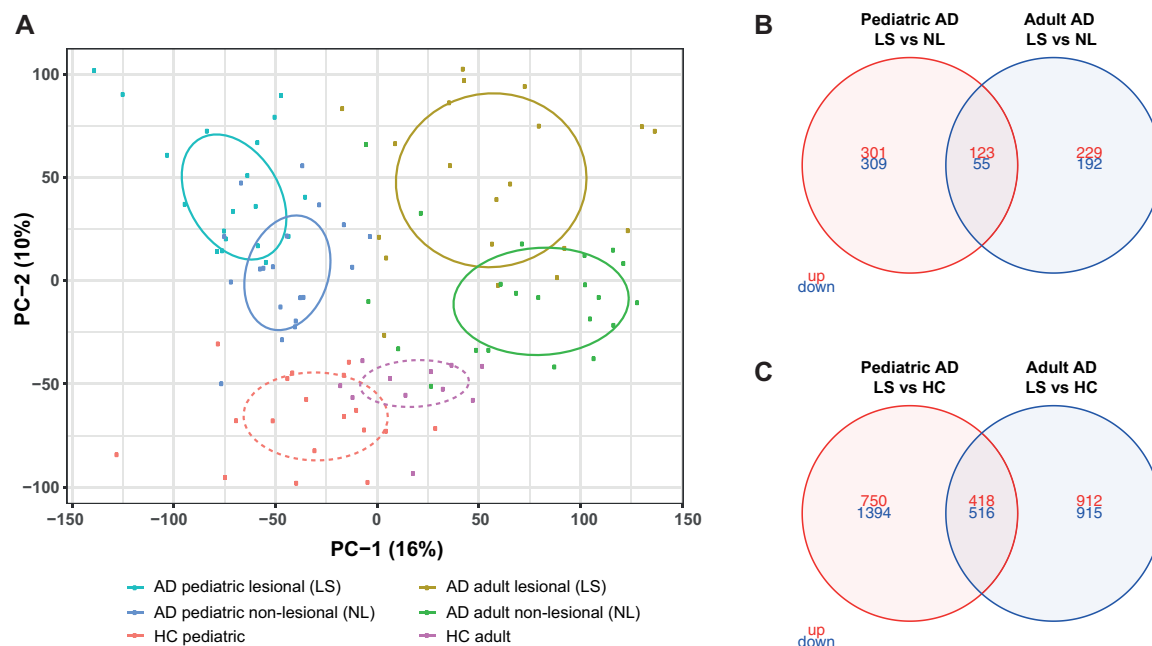


FIG 1. Transcriptional differences between pediatric and adult skin. **A**, A principal component (PC) analysis based on microarrays shows separate groupings of pediatric and adult AD (both lesional [LS] and nonlesional [NL]), as well as healthy control (HC) skin. **B** and **C**, Venn diagram of upregulated (red) and downregulated (blue) DEGs in lesional pediatric versus adult AD, either compared with respective nonlesional (Fig 1, B) or matched healthy control skin (Fig 1, C).

AD initiation, including allergic sensitization through the skin.^{13,14} The skin barrier is critically regulated by keratinocyte differentiation, including expression of genes of the epidermal differentiation complex (EDC), which includes genes encoding cornified envelope proteins.^{19,40} Other important barrier mediators are lipid metabolism components^{9,19,41–43} and proteins regulating tight and adherens junctions (eg, claudins [CLDNs] and cadherins).^{44,45} Differences in genomic expression of a predefined gene subset of barrier genes downregulated in adult AD^{16,19,27,41,46} were assessed in our AD groups compared with their respective control subjects. Markers with comparable expression levels in pediatric and adult healthy control samples are summarized in Fig 5 within (1) blue, (2) red, and (3) green boxes for genes preferentially downregulated in (1) pediatric AD, (2) both pediatric and adult AD, and (3) adult AD, respectively (Fig 5, A). Multiple genes encoding EDC/terminal differentiation proteins were significantly downregulated in nonlesional and, even more so, in lesional adult, but not in pediatric AD (Fig 5, A, gene names in orange letters); in contrast, several lipid-associated markers (Fig 5, A, gene names in green letters) showed significant reductions in both pediatric and/or adult skin.

Among important EDC and other differentiation-related genes that exhibited normal or near-normal expression in pediatric AD, but were significantly downregulated in adult AD, were *FLG*, *loricrin* (*LOR*), *scellin* (*SCEL*), and *PSORS1C2*,⁴⁷ as well as the late cornified envelope (LCE) proteins *LCE1B/LCE1E/LCE2B* (Fig 5, A). Conversely, annexin A6 (*ANXA6*), a calcium-dependent membrane and phospholipid-binding protein previously reported to be upregulated in adult AD and decreased in psoriasis,⁴⁸ was significantly downregulated in our pediatric patients (Fig 5, A). Levels of several adhesion molecules (CLDNs and cadherins) were decreased in both pediatric and adult AD,

including *CLDN8* and *CLDN23*, although to a greater extent in adult AD (Fig 5, A). *CLDN1*, which was previously shown to be reduced in adult AD and inversely correlated with serum IgE levels and eosinophil counts,⁴⁹ showed even stronger downregulation in pediatric AD.

Several lipid-related genes were also downregulated in pediatric AD. *ELOVL3* fatty acid elongase 3 (*ELOVL3*), a lipid-associated marker previously reported to be decreased in adult AD,⁴¹ was also significantly downregulated in pediatric AD (Fig 5, A, within the red box). *ELOVL3* encodes a protein involved in elongation of long-chain fatty acids that is essential in limiting TEWL.⁴¹ Another lipid mediator, diacylglycerol *o*-acyltransferase 2 (*DGAT2*), was also significantly downregulated in both adult and pediatric patients with AD. *DGAT2* knockout mice develop severe skin permeability barrier dysfunction and die from increased dehydration.⁵⁰ Other lipid barrier components, such as fatty acyl-CoA reductase 2 (*FAR2*) and fatty acid 2-hydroxylase (*FA2H*),⁴¹ showed preferential downregulation in pediatric AD (Fig 5, A).

Using RT-PCR, we assessed selected barrier-associated markers,^{19,41,51} such as *FAR2*, peroxisome proliferator activated receptor gamma (*PPARG*), and *LCE2B*, to confirm microarray results (Fig 5, B–D). Our data indicate that EDC/terminal differentiation downregulation, a phenomenon previously considered a hallmark of AD,^{52,53} was largely absent in our pediatric samples, whereas lipid and tight junction alterations characterized both adult and pediatric AD.

Further evidence of skin lipid defects in lesional and nonlesional pediatric AD

The stratum corneum serves as a platform for a neutral lipid enriched extracellular matrix.⁵⁴ This hydrophobic lipid matrix

[illegible]

FIG 2. Immune gene regulation in tissues from pediatric and adult patients with AD. Heat maps of immune-associated DEG subsets, as calculated by a fold change (*FCH*) of greater than 2 and false discovery rate (*FDR*) of less than 0.05 by using microarray data. Samples are sorted by means of hierarchical clustering: red, greater expression; blue, lower expression. Group comparisons (*FCH*s) are indicated. +*FDR*-adjusted $P < .1$; **FDR*-adjusted $P < .05$; ***FDR*-adjusted $P < .01$. *HC*, Age-matched healthy control subject; *LS*, lesional; *NL*, nonlesional. *Red box*, Upregulation in pediatric and adult patients with AD; *yellow box*, stronger upregulation in pediatric versus adult patients with AD; *blue box*, stronger upregulation in adult patients with AD; *green box*, preferential upregulation in pediatric patients with AD.

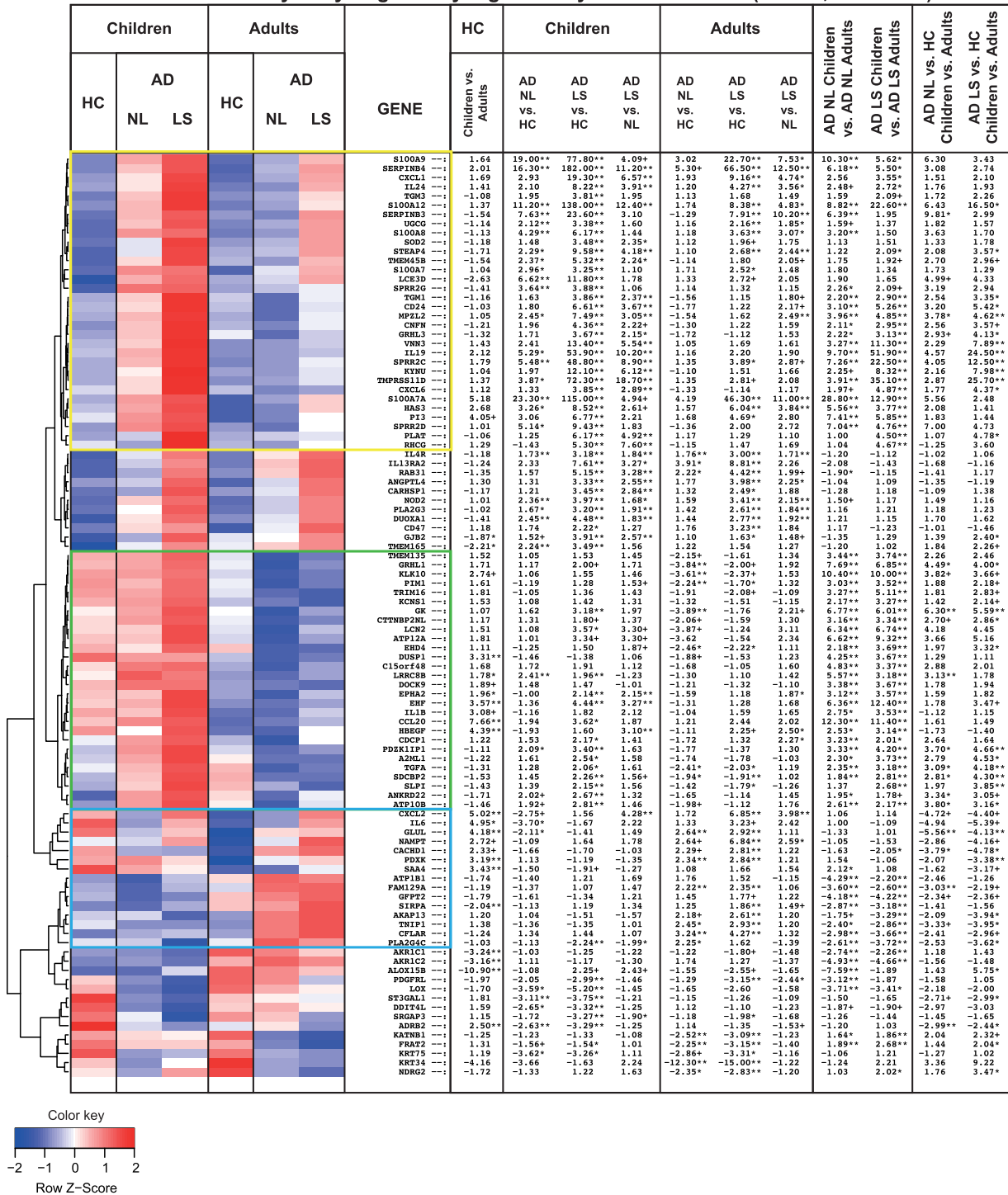
Genes additively or synergistically regulated by IL-17 and TNF- α (FCH>2, FDR<0.05)

FIG 3. Heat map of TNF- α - and/or IL-17-regulated DEG subsets, as calculated by a fold change (FCH) of greater than 2 and a false discovery rate (FDR) of less than 0.05 based on microarray data. Samples are sorted by using hierarchical clustering: red, greater expression; blue, lower expression. Group comparisons (FCHs) are indicated. +FDR-adjusted $P < .1$; *FDR-adjusted $P < .05$; **FDR-adjusted $P < .01$. HC, Age-matched healthy

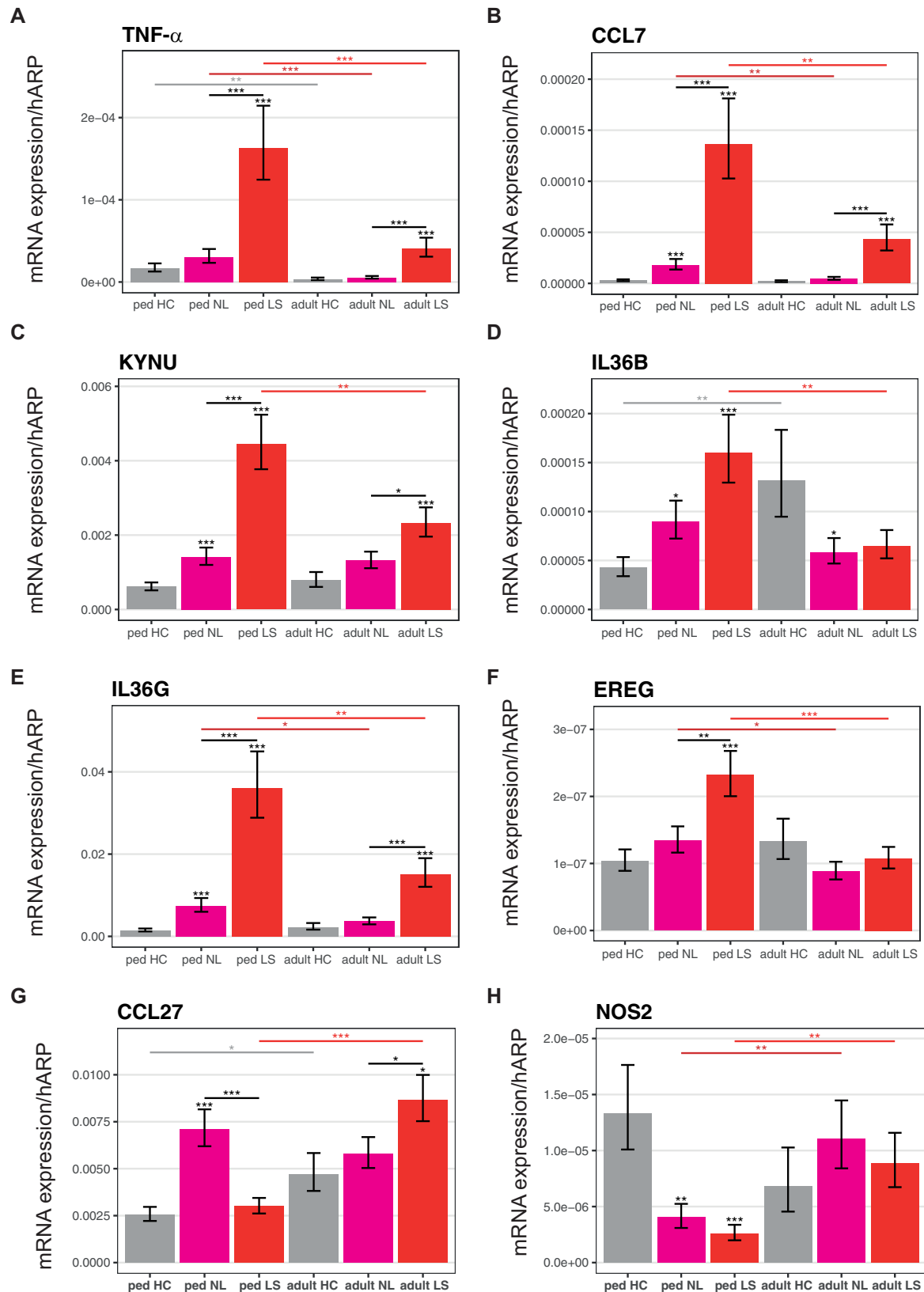
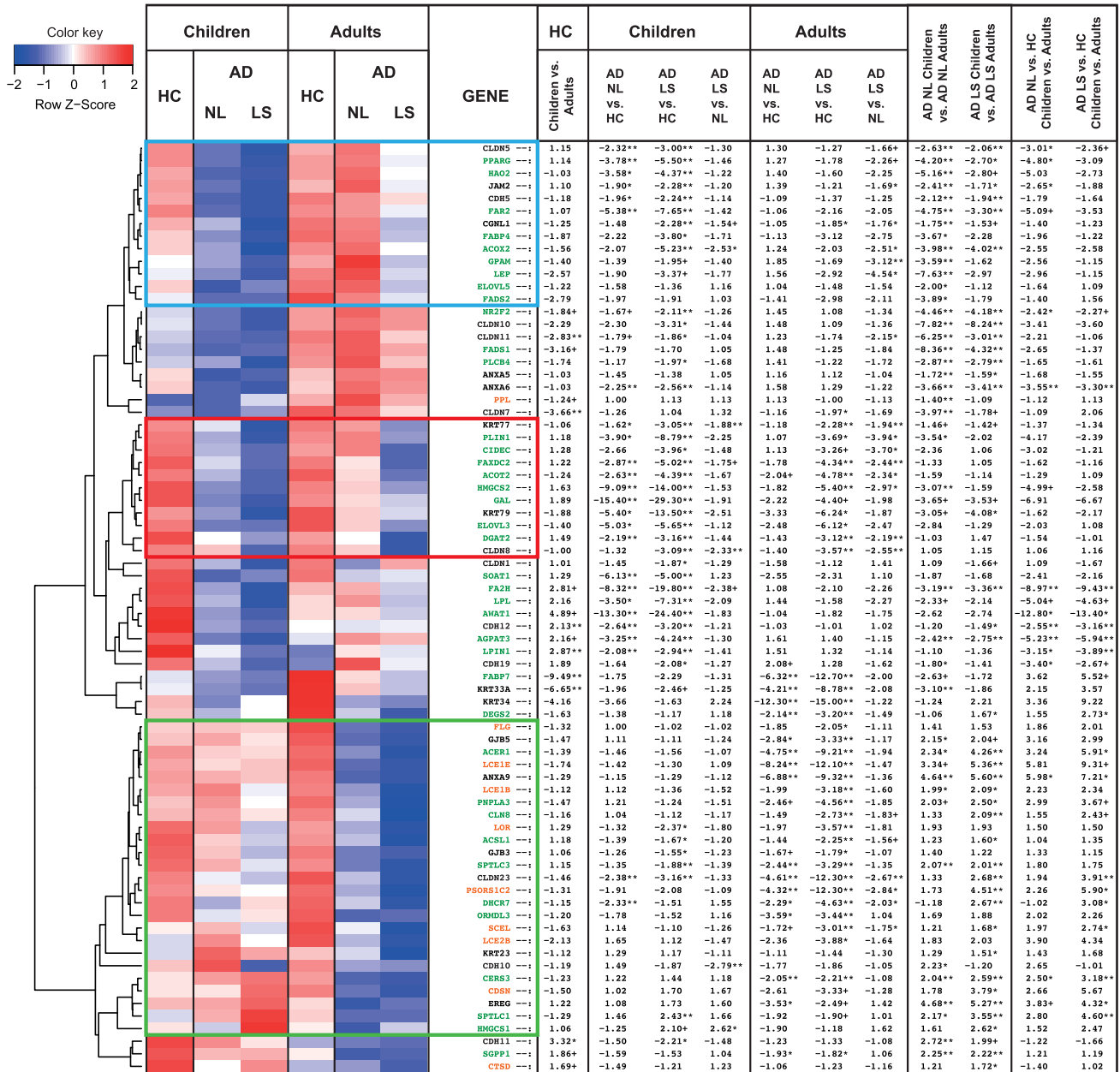
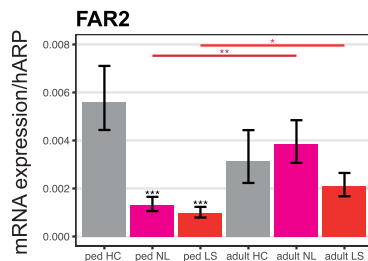


FIG 4. RT-PCR analysis (mRNA expression/human acidic ribosomal protein [*hARP*]) of pediatric and adult skin with matched healthy control subjects of disease markers previously reported to be strongly increased in psoriasis (A-F) and of genes previously described by Eyerich et al in a psoriasis/AD disease classifier (G and H): * $P < .05$, ** $P < .01$, and *** $P < .001$. Asterisks above error bars indicate a significant change from age-matched healthy control tissue; asterisks above lines refer to respective comparisons. HC, Healthy control subject; LS, lesional AD; NL, nonlesional AD; ped, pediatric.

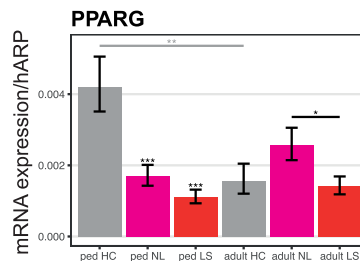
A

Barrier genes ($FCH > 2$, $FDR < 0.05$)

B



C



D

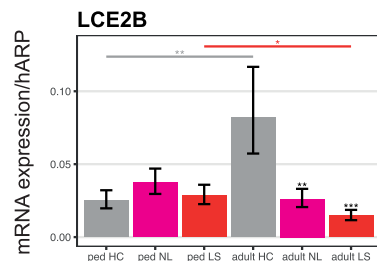


FIG 5. Skin barrier gene regulation in tissues from pediatric and adult patients with AD. **A**, Heat map of skin barrier-associated genes (including EDC/terminal differentiation marker genes in orange letters and lipid-associated marker genes in green letters), as calculated based on fold change (FCH) of greater than 2 and false discovery rate (FDR) of less than 0.05 based on microarray data. Samples are sorted by using

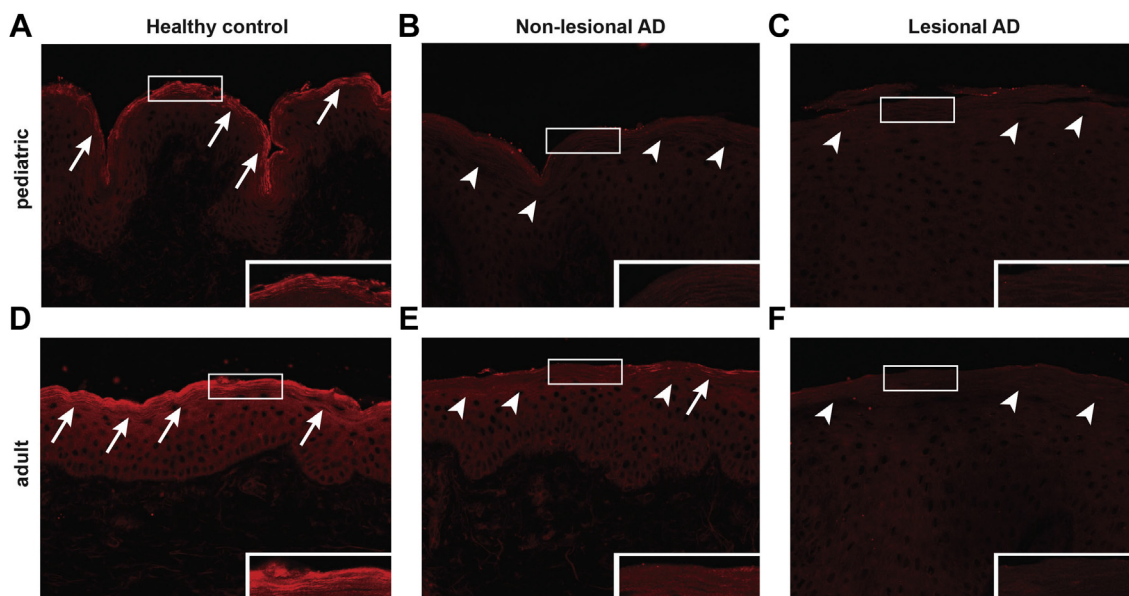


FIG 6. Skin lipid stain. Nile red stains of pediatric (A-C) and adult (D-F) samples of healthy control subjects and patients with lesional and nonlesional AD. Arrows, Organized lipid layer in stratum corneum; arrowheads, absence/near absence of organized lipid layer. Inset shows representative detail of the stratum corneum for each group.

enables the skin barrier to block the movement of water and electrolytes.⁵⁴ Because multiple lipid-associated mediators were downregulated in both pediatric and adult AD, we visualized stratum corneum lipids using Nile red staining.^{55,56} Although we observed a regularly layered staining pattern in the stratum corneum of healthy control skin (Fig 6, A, arrows), lipid layers were absent in both lesional and nonlesional AD pediatric skin (Fig 6, B and C, arrowheads) as in adult AD (Fig 6, D-F).¹⁹ These lipid alterations further explain the increases in TEWL, representing a functional barrier defect, in early-onset pediatric AD skin (Table I).

IL-26 shows a strong positive correlation with TEWL in pediatric AD

We next assessed the correlation of differentially regulated genes in pediatric AD tissues with TEWL, a functional measure of poor skin barrier integrity (see Table E2 in this article's Online Repository at www.jacionline.org).⁵⁷ The strongest positive correlation was found for IL-26 (Fig 7, A), an IL-20 family cytokine that signals through STAT1 and STAT3 and is strongly expressed by T_H17 cells.⁵⁸ IL-26 levels were increased significantly in nonlesional and lesional pediatric AD and, to a lesser extent, in lesional adult AD skin (Fig 2, within yellow box). Similarly, STAT3 expression (increased in pediatric vs adult AD, Fig 2, just below the green box) positively correlated with TEWL (Fig 7, B), whereas lipid-associated measures

downregulated in pediatric AD (eg, DAT2 and FA2H)^{41,50} showed negative correlations with TEWL (Fig 7, C and D, and see Table E2). These data further corroborate the notion that immune activation and skin lipid defects negatively affect the skin barrier in early-onset AD.

We also evaluated correlations between levels of total serum IgE and various skin markers. Serum IgE levels were positively correlated with some T_H2-associated mediators (TSLP and CCL27) and phosphodiesterase 4B (PDE4B), which is targeted by crisaborole,^{59,60} and negatively associated with barrier markers (ie, DGAT2 [lesional], FAR2 and CLD10 [nonlesional]; see Fig E1 and Table E3 in this article's Online Repository at www.jacionline.org).

DISCUSSION

This is the first study to broadly characterize immune and barrier properties in skin biopsy specimens from infants with recent-onset AD. Skin sensitization during early-onset pediatric AD is considered the initiating step of the atopic march,⁶¹ and its characterization might help develop approaches that can prevent atopic diathesis through either restoration of barrier integrity or early immune modulation.⁶² The pattern of immune activation shown by our data corroborates and expands the previous findings from our group based on analysis of a limited series of markers (ie, T_H2-centered inflammation in both adult and pediatric patients with AD, with stronger T_H9, T_H17, and T_H22 activation

hierarchical clustering: red, higher expression; blue, lower expression. Group comparisons (FCHs) are indicated. +FDR-adjusted $P < .1$; *FDR-adjusted $P < .05$; **FDR-adjusted $P < .01$. HC, Age-matched healthy control subject; LS, lesional; NL, nonlesional. Boxes indicate preferential downregulation in pediatric patients with AD (blue), both pediatric and adult patients with AD (red), and adult patients with AD (green). B-D, RT-PCR analysis of selected barrier markers. * $P < .05$, ** $P < .01$, and *** $P < .01$. Asterisks above error bars indicate significant difference from age-matched healthy control tissue. HC, Healthy control subjects; NL, nonlesional AD; LS, lesional AD; ped, pediatric.

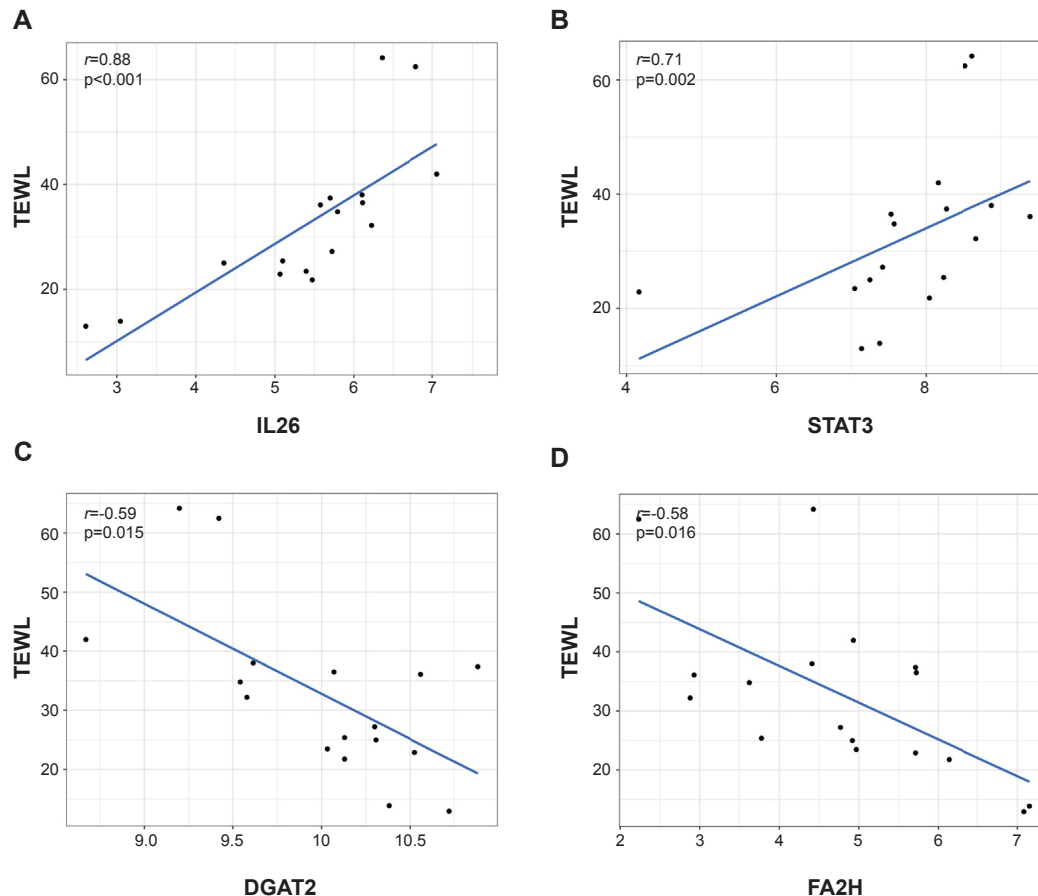


FIG 7. Scatter plots with TEWL (in grams per square meter per hour) and the indicated immune (A and B) and lipid-associated (C and D) mediators from microarray data from pediatric AD skin. r , Spearman correlation coefficient.

and lack of IFN- γ /Th1-associated inflammation in pediatric versus adult patients with AD).¹⁰

Importantly, we also explored the lack of downregulation of FLG expression because we still noted a functional barrier defect in our early-onset pediatric cohort, which was reflected by increased TEWL in lesional and nonlesional AD when compared with healthy children. To date, defects in FLG caused by downregulation by Th2 cytokines⁶³ or loss-of-function mutations, with the latter being found in 10% to 50% of patients with AD,⁶⁴ have been regarded as central to the barrier impairment seen in AD. FLG has been shown to be important for maintenance of epidermal homeostasis,^{65,66} and loss-of-function mutations in the *FLG* gene significantly increase the risk for AD and atopic diathesis.⁶⁷⁻⁶⁹ Lack of FLG has been demonstrated to disturb keratinocyte maturation and differentiation⁷⁰⁻⁷² and to negatively affect lipid composition⁷³ and natural moisturizing factors,^{74,75} and to increase skin permeability.⁷⁶ Common *FLG* mutations were absent in our patients,¹⁰ and we confirmed our prior findings of intact FLG expression in early-onset AD tissues using RT-PCR and immunohistochemistry staining.¹⁰ Similarly, other EDC/terminal differentiation mediators, including loricrin (LOR), the main component of the cornified envelope,⁷⁷ were downregulated to a very small extent in pediatric versus adult AD skin, despite the high TEWL levels in children with AD. These results

challenge the current dogma that EDC/terminal differentiation components are key pathogenic factors for the elicitation of AD.^{52,53} Because IL-4 and IL-13 have been shown to decrease FLG expression levels *in vitro*,⁶³ it was surprising that the strong Th2 polarization in pediatric patients with AD does not reduce expression of *FLG* and other EDC/terminal differentiation genes. Although earlier reports showed that IL-4 and IL-13, but not IFN- γ , inhibit FLG expression during keratinocyte differentiation *in vitro*,^{63,78} more recent studies demonstrated that IFN- γ is also able to downregulate FLG,^{79,80} as well as reduce ceramides and long-chain fatty acids,⁸¹ in human keratinocytes, potentially contributing to the accentuated differentiation abnormalities seen in adults versus children with AD.^{79,81,82}

Our data would suggest that abnormal barrier function in the skin of pediatric patients with AD does not reflect reduced differentiation but rather is suggestive of defects in tight junction proteins and lipid deposition, with the latter demonstrated by the virtually absent Nile red staining of the stratum corneum of lesional and nonlesional tissues from both pediatric and adult patients with AD. Since removal of stratum corneum lipids by using a gentle lipid solvent is sufficient to vastly abrogate the permeability barrier,^{83,84} the defect in the lipids within the stratum corneum may be the major contributor for the TEWL abnormalities observed in early-onset pediatric AD, as also suggested by the reductions in several genes involved in

regulation of barrier lipids in pediatric AD skin. Evaluation of lipid profiles by using mass spectrometry in early pediatric AD will thus be important to further delineate deficits in specific epidermal lipids. Both IL-17⁸⁵⁻⁸⁷ (increased in pediatric, but not adult AD skin) and T_H2 cytokines⁸⁸⁻⁹¹ were reported to negatively affect expression of tight junction components and permeability, as well as expression of CLDNs (1/8/23), which are key components of tight junctions, which were downregulated in both adult and pediatric AD tissues. Tight junction deficits might be particularly relevant in allowing permeation of larger xenobiotics, particularly when the overlying lipid-based barrier is also defective.⁸³

The strongly positive correlation of the T_H17 cytokine IL-26⁹² in pediatric lesional AD skin with TEWL, and negative correlations between TEWL and certain lipid-associated mediators, indicate a role for the T_H17 T-cell axis in creating the barrier properties of early-onset AD, which should be further explored. IL-26 has also been demonstrated to complex with bacterial and self-DNA to trigger Toll-like receptor 9 in plasmacytoid dendritic cells, potentially triggering skin inflammation.⁵⁸ IL-26 is also strongly upregulated in psoriasis and mycosis fungoides.⁹³ In contrast to IL-17, IL-26 expression is insensitive to inhibition by IL-4,⁹³ potentially explaining its high levels in our pediatric cohort despite strong T_H2 activation.

Levels of IFN- γ , which is considered a hallmark of chronic but not acute adult AD,⁹⁴ were not increased in early-onset pediatric skin, even though the pediatric AD lesions were chronic lesions (ie, >72 hours in duration). Consistently, pediatric lesions showed similar acanthosis to the skin of adult patients with chronic AD.¹⁰ In fact, some mediators of pediatric T_H1 cytokine expression are reduced in blood from pediatric patients with AD versus that from healthy subjects, such as IFN- γ ⁺ cutaneous lymphocyte antigen (CLA)⁺ skin-homing CD4⁺ T cells.⁹⁵ Despite this lack of IFN- γ responses, early-onset AD shares molecular features with psoriasis, as reflected by the significant increases in TNF- α /IL-17-associated markers, including classic psoriasis markers, such as EREG, KYNU, and antimicrobial peptides.^{10,12,35,38}

Nevertheless, a molecular psoriasis/AD disease classifier consisting of CCL27 and inducible nitric oxide synthase (NOS2)^{7,39} clearly categorized our pediatric samples as AD, similar to their adult AD counterparts. Interestingly, adult patients with AD showed progressive CCL27 increases from nonlesional to lesional AD, whereas in pediatric samples CCL27 levels were greater in patients with nonlesional versus lesional AD, which might be explained by the fact that increased IL-17 can downregulate CCL27 expression.^{39,96} CCL27 levels also positively correlated with serum IgE levels, which showed negative correlations with some barrier products, but these data were derived from a limited set of patients with available IgEs and require confirmation in larger studies.

This study sheds new light on the unique properties of early onset pediatric AD skin, highlighting the role of lipid abnormalities and IL-17/TNF- α -dependent inflammation, including expression of IL-26, as distinct features from adult AD skin. IFN- γ responses are likely to contribute to barrier defects, particularly those involving EDC/terminal differentiation, which predominate in adult AD lesions after years to decades of disease chronicity. The phenomenon of increased T_H1 and decreased T_H17 immune activation in adults with chronic AD may be rooted in mutual antagonism of T_H17

and IL-12/T_H1 responses, as observed in several model systems.^{97,98} However, whether the observed immune changes are truly causative of the pathology in patients with AD and the exact mechanism by which the transition from the early-onset pediatric to adult AD phenotype occurs or whether any of these mechanisms are involved in the determination of whether a child will outgrow AD or have persistent disease into adulthood remain to be elucidated. Also, ethnic differences among pediatric populations might influence the immune phenotype, as demonstrated in adult patients with AD,¹⁸ necessitating further investigation in larger pediatric cohorts. Similarly, the pediatric AD phenotype might be different in the setting of *FLG* loss-of-function mutations, which show type 1 interferon-mediated stress response, although in adolescents.⁹ Nevertheless, the distinct phenotype of early-onset pediatric AD highlighted by the present study might have profound implications for future targeted treatment approaches in this patient group.

Clinical implications: Numerous differences in immune (T_H17/T_H22/T_H1 responses) and barrier abnormalities (epidermal terminal differentiation) between early-onset pediatric AD versus longstanding adult AD might necessitate different targeted treatment approaches for these age groups.

REFERENCES

- Flohr C, Mann J. New insights into the epidemiology of childhood atopic dermatitis. *Allergy* 2014;69:3-16.
- Weidinger S, Novak N. Atopic dermatitis. *Lancet* 2016;387:1109-22.
- Silverberg JI. Public health burden and epidemiology of atopic dermatitis. *Dermatol Clin* 2017;35:283-9.
- Werfel T, Allam JP, Biedermann T, Eyerich K, Gilles S, Guttman-Yassky E, et al. Cellular and molecular immunologic mechanisms in patients with atopic dermatitis. *J Allergy Clin Immunol* 2016;138:336-49.
- Leung DY, Guttman-Yassky E. Deciphering the complexities of atopic dermatitis: shifting paradigms in treatment approaches. *J Allergy Clin Immunol* 2014;134:769-79.
- Ewald DA, Noda S, Oliva M, Litman T, Nakajima S, Li X, et al. Major differences between human atopic dermatitis and murine models, as determined by using global transcriptomic profiling. *J Allergy Clin Immunol* 2017;139:562-71.
- Quaranta M, Knapp B, Garzorz N, Mattii M, Pullabhatla V, Pennino D, et al. Intraindividual genome expression analysis reveals a specific molecular signature of psoriasis and eczema. *Sci Transl Med* 2014;6:244ra90.
- Suarez-Farinas M, Ungar B, Correa da Rosa J, Ewald DA, Rozenblit M, Gonzalez J, et al. RNA sequencing atopic dermatitis transcriptome profiling provides insights into novel disease mechanisms with potential therapeutic implications. *J Allergy Clin Immunol* 2015;135:1218-27.
- Cole C, Kroboth K, Schurch NJ, Sandilands A, Sherstnev A, O'Regan GM, et al. Filaggrin-stratified transcriptomic analysis of pediatric skin identifies mechanistic pathways in patients with atopic dermatitis. *J Allergy Clin Immunol* 2014;134:82-91.
- Esaki H, Brunner PM, Renert-Yuval Y, Czarnowicki T, Huynh T, Tran G, et al. Early-onset pediatric atopic dermatitis is TH2 but also TH17 polarized in skin. *J Allergy Clin Immunol* 2016;138:1639-51.
- Gittler JK, Shemer A, Suarez-Farinas M, Fuentes-Duculan J, Gulewicz KJ, Wang CQ, et al. Progressive activation of T(H)2/T(H)22 cytokines and selective epidermal proteins characterizes acute and chronic atopic dermatitis. *J Allergy Clin Immunol* 2012;130:1344-54.
- Ong PY, Ohtake T, Brandt C, Strickland I, Boguniewicz M, Ganz T, et al. Endogenous antimicrobial peptides and skin infections in atopic dermatitis. *N Engl J Med* 2002;347:1151-60.
- Irvine AD, McLean WH, Leung DY. Filaggrin mutations associated with skin and allergic diseases. *N Engl J Med* 2011;365:1315-27.
- Pellerin L, Henry J, Hsu CY, Balica S, Jean-Decoster C, Mechin MC, et al. Defects of filaggrin-like proteins in both lesional and nonlesional atopic skin. *J Allergy Clin Immunol* 2013;131:1094-102.

15. Kelleher M, Dunn-Galvin A, Hourihane JO, Murray D, Campbell LE, McLean WH, et al. Skin barrier dysfunction measured by transepidermal water loss at 2 days and 2 months predates and predicts atopic dermatitis at 1 year. *J Allergy Clin Immunol* 2015;135:930-5.
16. Khattri S, Shemer A, Rozenblit M, Dhingra N, Czarnowicki T, Finney R, et al. Cyclosporine in patients with atopic dermatitis modulates activated inflammatory pathways and reverses epidermal pathology. *J Allergy Clin Immunol* 2014;133:1626-34.
17. Johnson-Huang LM, Suarez-Farinas M, Sullivan-Whalen M, Gilleaudeau P, Krueger JG, Lowes MA. Effective narrow-band UVB radiation therapy suppresses the IL-23/IL-17 axis in normalized psoriasis plaques. *J Invest Dermatol* 2010;130:2654-63.
18. Noda S, Suarez-Farinas M, Ungar B, Kim SJ, de Guzman Strong C, Xu H, et al. The Asian atopic dermatitis phenotype combines features of atopic dermatitis and psoriasis with increased TH17 polarization. *J Allergy Clin Immunol* 2015;136:1254-64.
19. Guttman-Yassky E, Suarez-Farinas M, Chiricozzi A, Nograles KE, Shemer A, Fuentes-Duculan J, et al. Broad defects in epidermal cornification in atopic dermatitis identified through genomic analysis. *J Allergy Clin Immunol* 2009;124:1235-44.e58s.
20. Suarez-Farinas M, Ungar B, Noda S, Shroff A, Mansouri Y, Fuentes-Duculan J, et al. Alopecia areata profiling shows TH1, TH2, and IL-23 cytokine activation without parallel TH17/TH22 skewing. *J Allergy Clin Immunol* 2015;136:1277-87.
21. Brunner PM, Khattri S, Garcet S, Finney R, Oliva M, Dutt R, et al. A mild topical steroid leads to progressive anti-inflammatory effects in the skin of patients with moderate-to-severe atopic dermatitis. *J Allergy Clin Immunol* 2016;138:169-78.
22. Suarez-Farinas M, Pellegrino M, Wittkowski KM, Magnasco MO. Harshlight: a "corrective make-up" program for microarray chips. *BMC Bioinformatics* 2005;6:294.
23. Wu Z, Irizarry R, Gentleman R, Martinez-Murillo F, Spencer F. A model-based background adjustment for oligonucleotide expression arrays. *J Am Stat Assoc* 2004;99:909-17.
24. Chen C, Grennan K, Badner J, Zhang D, Gershon E, Jin L, et al. Removing batch effects in analysis of expression microarray data: an evaluation of six batch adjustment methods. *PLoS One* 2011;6:e17238.
25. Brunner PM, Guttman-Yassky E, Leung DY. The immunology of atopic dermatitis and its reversibility with broad-spectrum and targeted therapies. *J Allergy Clin Immunol* 2017;139(suppl):S65-76.
26. Koga C, Kabashima K, Shiraishi N, Kobayashi M, Tokura Y. Possible pathogenic role of Th17 cells for atopic dermatitis. *J Invest Dermatol* 2008;128:2625-30.
27. Hamilton JD, Suarez-Farinas M, Dhingra N, Cardinale I, Li X, Kostic A, et al. Dupilumab improves the molecular signature in skin of patients with moderate-to-severe atopic dermatitis. *J Allergy Clin Immunol* 2014;134:1293-300.
28. Witte E, Kokolakis G, Witte K, Philipp S, Doecke WD, Babel N, et al. IL-19 is a component of the pathogenetic IL-23/IL-17 cascade in psoriasis. *J Invest Dermatol* 2014;134:2757-67.
29. Huang F, Wachi S, Thai P, Loukianov A, Tan KH, Forteza RM, et al. Potentiation of IL-19 expression in airway epithelia by IL-17A and IL-4/IL-13: important implications in asthma. *J Allergy Clin Immunol* 2008;121:1415-21, e1-3.
30. Abenius MS, Lempinen E, Lindblad K, Ernerudh J, Berg G, Matthiesen L, et al. Th2-like chemokine levels are increased in allergic children and influenced by maternal immunity during pregnancy. *Pediatr Allergy Immunol* 2014;25:387-93.
31. Kim J, Krueger JG. The immunopathogenesis of psoriasis. *Dermatol Clin* 2015;33:13-23.
32. Chiricozzi A, Guttman-Yassky E, Suarez-Farinas M, Nograles KE, Tian S, Cardinale I, et al. Integrative responses to IL-17 and TNF-alpha in human keratinocytes account for key inflammatory pathogenic circuits in psoriasis. *J Invest Dermatol* 2011;131:677-87.
33. Guttman-Yassky E, Lowes MA, Fuentes-Duculan J, Whynot J, Novitskaya I, Cardinale I, et al. Major differences in inflammatory dendritic cells and their products distinguish atopic dermatitis from psoriasis. *J Allergy Clin Immunol* 2007;119:1210-7.
34. Brunner PM, Glitzner E, Reininger B, Klein I, Stary G, Mildner M, et al. CCL7 contributes to the TNF-alpha-dependent inflammation of lesional psoriatic skin. *Exp Dermatol* 2015;24:522-8.
35. Harden JL, Lewis SM, Lish SR, Suarez-Farinas M, Gareau D, Lentini T, et al. The tryptophan metabolism enzyme L-kynureninase is a novel inflammatory factor in psoriasis and other inflammatory diseases. *J Allergy Clin Immunol* 2016;137:1830-40.
36. Johnston A, Xing X, Guzman AM, Riblett M, Loyd CM, Ward NL, et al. IL-1F5, -F6, -F8, and -F9: a novel IL-1 family signaling system that is active in psoriasis and promotes keratinocyte antimicrobial peptide expression. *J Immunol* 2011;186:2613-22.
37. D'Erme AM, Wilschmann-Theis D, Wagenpfeil J, Holzel M, Ferring-Schmitt S, Sternberg S, et al. IL-36gamma (IL-1F9) is a biomarker for psoriasis skin lesions. *J Invest Dermatol* 2015;135:1025-32.
38. Shirakata Y, Kishimoto J, Tokumaru S, Yamasaki K, Hanakawa Y, Tohyama M, et al. Epiregulin, a member of the EGF family, is over-expressed in psoriatic epidermis. *J Dermatol Sci* 2007;45:69-72.
39. Garzorz N, Eyerich K. NOS2 and CCL27: clinical implications for psoriasis and eczema diagnosis and management. *Expert Rev Clin Immunol* 2015;11:167-9.
40. Sugiura H, Ebise H, Tazawa T, Tanaka K, Sugiura Y, Uehara M, et al. Large-scale DNA microarray analysis of atopic skin lesions shows overexpression of an epidermal differentiation gene cluster in the alternative pathway and lack of protective gene expression in the cornified envelope. *Br J Dermatol* 2005;152:146-9.
41. Ewald DA, Malajian D, Krueger JG, Workman CT, Wang T, Tian S, et al. Meta-analysis derived atopic dermatitis (MADAD) transcriptome defines a robust AD signature highlighting the involvement of atherosclerosis and lipid metabolism pathways. *BMC Med Genomics* 2015;8:60.
42. Saki H, Ewald DA, Ungar B, Rozenblit M, Zheng X, Xu H, et al. Identification of novel immune and barrier genes in atopic dermatitis by means of laser capture microdissection. *J Allergy Clin Immunol* 2015;135:153-63.
43. Mathay C, Pierre M, Pittelkow MR, Depiereux E, Nikkels AF, Colige A, et al. Transcriptional profiling after lipid raft disruption in keratinocytes identifies critical mediators of atopic dermatitis pathways. *J Invest Dermatol* 2011;131:46-58.
44. Zaniboni MC, Samorano LP, Orfali RL, Aoki V. Skin barrier in atopic dermatitis: beyond filaggrin. *An Bras Dermatol* 2016;91:472-8.
45. Ishida-Yamamoto A, Igawa S, Kishibe M. Order and disorder in corneocyte adhesion. *J Dermatol* 2011;38:645-54.
46. Tintle S, Shemer A, Suarez-Farinas M, Fujita H, Gilleaudeau P, Sullivan-Whalen M, et al. Reversal of atopic dermatitis with narrow-band UVB phototherapy and biomarkers for therapeutic response. *J Allergy Clin Immunol* 2011;128:583-93, e1-4.
47. Abbas Zadeh S, Mlitz V, Lachner J, Golabi B, Mildner M, Pammer J, et al. Phylogenetic profiling and gene expression studies implicate a primary role of PSORS1C2 in terminal differentiation of keratinocytes. *Exp Dermatol* 2017;26:352-8.
48. Baurecht H, Hotze M, Brand S, Buning C, Cormican P, Corvin A, et al. Genome-wide comparative analysis of atopic dermatitis and psoriasis gives insight into opposing genetic mechanisms. *Am J Hum Genet* 2015;96:104-20.
49. De Benedetto A, Rafaels NM, McGirt LY, Ivanov AI, Georas SN, Cheadle C, et al. Tight junction defects in patients with atopic dermatitis. *J Allergy Clin Immunol* 2011;127:773-86, e1-7.
50. Radner FP, Fischer J. The important role of epidermal triacylglycerol metabolism for maintenance of the skin permeability barrier function. *Biochim Biophys Acta* 2014;1841:409-15.
51. Kuenzli S, Saurat JH. Effect of topical PPARbeta/delta and PPARgamma agonists on plaque psoriasis. A pilot study. *Dermatology* 2003;206:252-6.
52. Cabanillas B, Novak N. Atopic dermatitis and filaggrin. *Curr Opin Immunol* 2016;42:1-8.
53. Bin L, Leung DY. Genetic and epigenetic studies of atopic dermatitis. *Allergy Asthma Clin Immunol* 2016;12:52.
54. Feingold KR, Elias PM. Role of lipids in the formation and maintenance of the cutaneous permeability barrier. *Biochim Biophys Acta* 2014;1841:280-94.
55. Lee JN, Jee SH, Chan CC, Lo W, Dong CY, Lin SJ. The effects of depilatory agents as penetration enhancers on human stratum corneum structures. *J Invest Dermatol* 2008;128:2240-7.
56. Sheu HM, Tsai JC, Lin TK, Wong TW, Lee JY. Modified Nile red staining method for improved visualization of neutral lipid depositions in stratum corneum. *J Formos Med Assoc* 2003;102:656-60.
57. Kottner J, Lichterfeld A, Blume-Peytavi U. Transepidermal water loss in young and aged healthy humans: a systematic review and meta-analysis. *Arch Dermatol Res* 2013;305:315-23.
58. Meller S, Di Domizio J, Voo KS, Friedrich HC, Chamilos G, Ganguly D, et al. T(H)17 cells promote microbial killing and innate immune sensing of DNA via interleukin 26. *Nat Immunol* 2015;16:970-9.
59. Paller AS, Tom WL, Lebwohl MG, Blumenthal RL, Boguniewicz M, Call RS, et al. Efficacy and safety of crisaborole ointment, a novel, nonsteroidal phosphodiesterase 4 (PDE4) inhibitor for the topical treatment of atopic dermatitis (AD) in children and adults. *J Am Acad Dermatol* 2016;75:494-503.e4.
60. Paller AS, Kabashima K, Bieber T. Therapeutic pipeline for atopic dermatitis: end of the drought? *J Allergy Clin Immunol* 2017;140:633-43.
61. Han H, Roan F, Ziegler SF. The atopic march: current insights into skin barrier dysfunction and epithelial cell-derived cytokines. *Immunol Rev* 2017;278:116-30.
62. Czarnowicki T, Krueger JG, Guttman-Yassky E. Novel concepts of prevention and treatment of atopic dermatitis through barrier and immune manipulations

- with implications for the atopic march. *J Allergy Clin Immunol* 2017;139:1723-34.
63. Howell MD, Kim BE, Gao P, Grant AV, Boguniewicz M, DeBenedetto A, et al. Cytokine modulation of atopic dermatitis filaggrin skin expression. *J Allergy Clin Immunol* 2007;120:150-5.
 64. Kim BE, Leung DY. Epidermal barrier in atopic dermatitis. *Allergy Asthma Immunol Res* 2012;4:12-6.
 65. Brown SJ, McLean WH. One remarkable molecule: filaggrin. *J Invest Dermatol* 2012;132:751-62.
 66. Presland RB, Coulombe PA, Eckert RL, Mao-Qiang M, Feingold KR, Elias PM. Barrier function in transgenic mice overexpressing K16, involucrin, and filaggrin in the suprabasal epidermis. *J Invest Dermatol* 2004;123:603-6.
 67. Brown SJ, McLean WH. Eczema genetics: current state of knowledge and future goals. *J Invest Dermatol* 2009;129:543-52.
 68. Palmer CN, Irvine AD, Terron-Kwiatkowski A, Zhao Y, Liao H, Lee SP, et al. Common loss-of-function variants of the epidermal barrier protein filaggrin are a major predisposing factor for atopic dermatitis. *Nat Genet* 2006;38:441-6.
 69. Zheng T, Yu J, Oh MH, Zhu Z. The atopic march: progression from atopic dermatitis to allergic rhinitis and asthma. *Allergy Asthma Immunol Res* 2011;3:67-73.
 70. Kuchler S, Henkes D, Eckl KM, Ackermann K, Plendl J, Korting HC, et al. Hallmarks of atopic skin mimicked in vitro by means of a skin disease model based on FLG knock-down. *Altern Lab Anim* 2011;39:471-80.
 71. Mildner M, Jin J, Eckhart L, Kezic S, Gruber F, Barresi C, et al. Knockdown of filaggrin impairs diffusion barrier function and increases UV sensitivity in a human skin model. *J Invest Dermatol* 2010;130:2286-94.
 72. Pendaries V, Malaise J, Pellerin L, Le Lamer M, Nachat R, Kezic S, et al. Knockdown of filaggrin in a three-dimensional reconstructed human epidermis impairs keratinocyte differentiation. *J Invest Dermatol* 2014;134:2938-46.
 73. Vavrova K, Henkes D, Struver K, Sochorova M, Skolova B, Witting MY, et al. Filaggrin deficiency leads to impaired lipid profile and altered acidification pathways in a 3D skin construct. *J Invest Dermatol* 2014;134:746-53.
 74. Janssens M, van Smeden J, Gooris GS, Bras W, Portale G, Caspers PJ, et al. Increase in short-chain ceramides correlates with an altered lipid organization and decreased barrier function in atopic eczema patients. *J Lipid Res* 2012;53:2755-66.
 75. Kezic S, O'Regan GM, Lutter R, Jakasa I, Koster ES, Saunders S, et al. Filaggrin loss-of-function mutations are associated with enhanced expression of IL-1 cytokines in the stratum corneum of patients with atopic dermatitis and in a murine model of filaggrin deficiency. *J Allergy Clin Immunol* 2012;129:1031-9.e1.
 76. Gruber R, Elias PM, Crumrine D, Lin TK, Brandner JM, Hachem JP, et al. Filaggrin genotype in ichthyosis vulgaris predicts abnormalities in epidermal structure and function. *Am J Pathol* 2011;178:2252-63.
 77. Jarnik M, Simon MN, Steven AC. Cornified cell envelope assembly: a model based on electron microscopic determinations of thickness and projected density. *J Cell Sci* 1998;111:1051-60.
 78. Howell MD, Fairchild HR, Kim BE, Bin L, Boguniewicz M, Redzic JS, et al. Th2 cytokines act on S100/A11 to downregulate keratinocyte differentiation. *J Invest Dermatol* 2008;128:2248-58.
 79. Noh M, Yeo H, Ko J, Kim HK, Lee CH. MAP17 is associated with the T-helper cell cytokine-induced down-regulation of filaggrin transcription in human keratinocytes. *Exp Dermatol* 2010;19:355-62.
 80. Hvid M, Johansen C, Deleuran B, Kemp K, Deleuran M, Vestergaard C. Regulation of caspase 14 expression in keratinocytes by inflammatory cytokines—a possible link between reduced skin barrier function and inflammation? *Exp Dermatol* 2011;20:633-6.
 81. Tawada C, Kanoh H, Nakamura M, Mizutani Y, Fujisawa T, Banno Y, et al. Interferon-gamma decreases ceramides with long-chain fatty acids: possible involvement in atopic dermatitis and psoriasis. *J Invest Dermatol* 2014;134:712-8.
 82. Feingold KR. The adverse effect of IFN gamma on stratum corneum structure and function in psoriasis and atopic dermatitis. *J Invest Dermatol* 2014;134:597-600.
 83. Elias PM, Wakefield JS. Mechanisms of abnormal lamellar body secretion and the dysfunctional skin barrier in patients with atopic dermatitis. *J Allergy Clin Immunol* 2014;134:781-91.e1.
 84. Grubauer G, Feingold KR, Harris RM, Elias PM. Lipid content and lipid type as determinants of the epidermal permeability barrier. *J Lipid Res* 1989;30:89-96.
 85. Gutowska-Owsiak D, Ogg GS. Cytokine regulation of the epidermal barrier. *Clin Exp Allergy* 2013;43:586-98.
 86. Yuki T, Tobishi M, Kusaka-Kikushima A, Ota Y, Tokura Y. Impaired tight junctions in atopic dermatitis skin and in a skin-equivalent model treated with interleukin-17. *PLoS One* 2016;11:e0161759.
 87. Gutowska-Owsiak D, Schaupp AL, Salimi M, Selvakumar TA, McPherson T, Taylor S, et al. IL-17 downregulates filaggrin and affects keratinocyte expression of genes associated with cellular adhesion. *Exp Dermatol* 2012;21:104-10.
 88. Gruber R, Bornchen C, Rose K, Daubmann A, Volksdorf T, Wladykowski E, et al. Diverse regulation of claudin-1 and claudin-4 in atopic dermatitis. *Am J Pathol* 2015;185:2777-89.
 89. Brandner JM, Zorn-Kruppa M, Yoshida T, Moll I, Beck LA, De Benedetto A. Epidermal tight junctions in health and disease. *Tissue Barriers* 2015;3:e974451.
 90. Omori-Miyake M, Yamashita M, Tsunemi Y, Kawashima M, Yagi J. In vitro assessment of IL-4- or IL-13-mediated changes in the structural components of keratinocytes in mice and humans. *J Invest Dermatol* 2014;134:1342-50.
 91. Kuo IH, De Benedetto A, Yoshida T, Leung DY, Beck LA. T helper cytokines (Th1, Th2 and Th17) differentially regulate the epidermal tight junction barrier. Presented at: AAAAI Annual Meeting; San Antonio, February 22-26, 2013.
 92. Pene J, Chevalier S, Preisser L, Venereau E, Guilleux MH, Ghannam S, et al. Chronically inflamed human tissues are infiltrated by highly differentiated Th17 lymphocytes. *J Immunol* 2008;180:7423-30.
 93. Wolk K, Mitsui H, Witte K, Gellrich S, Gulati N, Humme D, et al. Deficient cutaneous antibacterial competence in cutaneous T-cell lymphomas: role of Th2-mediated biased Th17 function. *Clin Cancer Res* 2014;20:5507-16.
 94. Yamanaka K, Mizutani H. The role of cytokines/chemokines in the pathogenesis of atopic dermatitis. *Curr Probl Dermatol* 2011;41:80-92.
 95. Czarnowicki T, Esaki H, Gonzalez J, Malajian D, Shemer A, Noda S, et al. Early pediatric atopic dermatitis shows only a cutaneous lymphocyte antigen (CLA)(+) TH2/TH1 cell imbalance, whereas adults acquire CLA(+) TH22/TC22 cell subsets. *J Allergy Clin Immunol* 2015;136:941-51.e3.
 96. Kanda N, Koike S, Watanabe S. IL-17 suppresses TNF-alpha-induced CCL27 production through induction of COX-2 in human keratinocytes. *J Allergy Clin Immunol* 2005;116:1144-50.
 97. Damsker JM, Hansen AM, Caspi RR. Th1 and Th17 cells: adversaries and collaborators. *Ann N Y Acad Sci* 2010;1183:211-21.
 98. Kulig P, Musiol S, Freiburger SN, Schreiner B, Gyulveszi G, Russo G, et al. IL-12 protects from psoriasisiform skin inflammation. *Nat Commun* 2016;7:13466.

Early-onset pediatric atopic dermatitis is characterized by Th2/Th17/Th22-centered inflammation and lipid alterations

Supplementary Methods

Biomarker evaluations

Total serum IgE was measured using the ImmunoCAP assay (Thermo Fisher Scientific). Due to scarcity of sample, total serum IgE levels were only available for 8 patients.

Supplementary Figure Legends

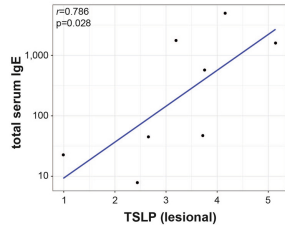
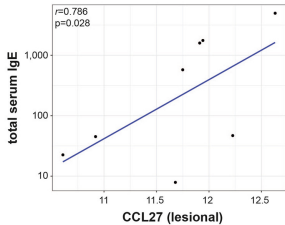
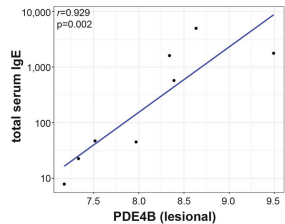
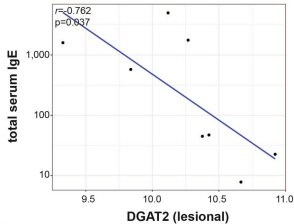
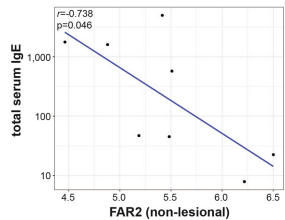
Supplementary Figure E1. Scatter plots correlating levels of total serum IgE (U/mL) with the indicated lesional **(A-D)** and non-lesional measures **(E-F)** from microarray data; *r* Spearman correlation coefficient.

Supplementary Table Legends

Supplementary Table E1. Excel file of differentially regulated genes in microarray analyses in pediatric and adult samples.

Supplementary Table E2. Excel file of correlation of transepidermal water loss (TEWL) measures with microarray data from lesional pediatric AD skin.

Supplementary Table E3. Excel file of correlation of total serum IgE measures with microarray data from lesional and non-lesional pediatric AD skin.

A**B****C****D****E****F**