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Use of Tape Strips to Detect Immune and Barrier Abnormalities in the Skin of Children With Early-Onset Atopic Dermatitis

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IMPORTANCE Molecular profiling of skin biopsies is the criterion standard for evaluating the cutaneous atopic dermatitis (AD) phenotype. However, skin biopsies are not always feasible in children. A reproducible minimally invasive approach that can track cutaneous disease in pediatric longitudinal studies or clinical trials is lacking.

OBJECTIVE To assess a minimally invasive approach using tape strips to identify skin biomarkers that may serve as a surrogate to biomarkers identified using whole-tissue biopsies.

DESIGN, SETTING, AND PARTICIPANTS This cross-sectional study of 51 children younger than 5 years recruited children with moderate to severe AD and children without AD from the dermatology outpatient clinics at a children's hospital. Sixteen tape strips were serially collected from the nonlesional and lesional skin of 21 children who had AD and were less than 6 months from disease initiation and from the normal skin of 30 children who did not have AD between January 22, 2016, and April 20, 2018.

MAIN OUTCOMES AND MEASURES Gene and protein expression were evaluated using quantitative real-time polymerase chain reaction and immunohistochemistry.

RESULTS A total of 51 children younger than 5 years were included in the study; 21 children had moderate to severe AD with less than 6 months of disease duration, and 30 children did not have AD. Of the 21 children with AD, the mean (SD) age was 1.7 (1.7) years, and most were male (15 [71.4%] and white (15 [71.4%]). Of the 30 children without AD, the mean (SD) age was 1.8 (2.0) years, and most were female (20 [66.7%]) and white (22 [73.3%]). Seventy-seven of 79 evaluated immune and barrier gene products were detected (gene detection rate, 97%) in 70 of 71 tape strips (sample detection rate, 99%), with 53 of 79 markers differentiating between children with lesional and/or nonlesional AD from children without AD. Many cellular markers of T cells (CD3), AD-related dendritic cells (Fc ε RI and OX40 ligand receptors), and key inflammatory (matrix metallopeptidase 12), innate (interleukin 8 [IL-8] and IL-6), helper T cell 2 (T_H2; IL-4, IL-13, and chemokines CCL17 and CCL26), and T_H17/T_H22 (IL-19, IL-36G, and S100A proteins) genes were significantlyincreased in lesional and nonlesional AD compared with tape strips from normal skin. For example, IL-4 mean (SE) for lesional was −15.2 (0.91) and normal was −19.5 (0.48); $P < .001$. Parallel decreases occurred in epidermal barrier gene products (FLG, CLDN23, and FA2H) and negative immune regulators (IL-34 and IL-37). For example, the decrease for FLG lesional was mean (SE) −2.9 (0.42) and for normal was 2.2 (0.45); $P < .001$. Associations were found between disease severity or transepidermal water loss and T_H2 (IL-33 and IL-4R) and T_H17/T_H22 (IL-36G and S100As) products in lesional and nonlesional AD skin (evaluated using the SCORing Atopic Dermatitis, Eczema Area and Severity Index, and Pruritus Atopic Dermatitis Quickscore tools).

CONCLUSIONS AND RELEVANCE In this study, tape strips provide a minimally invasive alternative for serially evaluating AD-associated cutaneous biomarkers and may prove useful for tracking pediatric AD therapeutic response and predicting future course and comorbidities.

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Editorial
Supplemental content

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Atopic dermatitis (AD) has a growing therapeutic pipeline,[1-9] largely owing to increased understanding of AD mechanisms in adults.[2,6-20] Previous studies identified treatment-response biomarkers, which are important for understanding molecular tissue responses and their association with clinical severity.[6,15,16,18,20-23] However, AD onset usually occurs in children who are younger than 5 years.[24-26] Recently, AD-associated biomarkers were identified by whole-skin profiling from children younger than 5 years [27,28] and adolescents.[29] However, biopsies are not always practical in children, and much less invasive blood phenotyping cannot capture the complex AD skin phenotype.[30-35] Minimally invasive approaches that accurately capture key immune and barrier biomarkers in the skin of patients with early-onset pediatric AD are needed.

Research has been directed toward identifying AD biomarkers using tape strips, a minimally invasive method that captures the stratum corneum.[36-69] However, most tape-stripping studies have focused on adults with chronic AD,[45,47,52-54,56-58,63,66,70,71] studying a limited panel of protein analytes, including antimicrobials (human β-defensin 4 [hBD4] and antimicrobial peptide cathelicidin LL-37), serine proteases (kallikrein-related peptidase 5 [KLK5] and KLK7),[45,52,57,72] lipids,[46] and inflammatory proteins (interleukin 4 [IL-4] and chemokine 17 [CCL17]).[53,70]

Two recent pediatric studies[60,61] used proteomic immune assays to evaluate stratum corneum biomarkers in tape strips from the nonlesional skin of infants with moderate to severe AD compared with infants without AD[61] and from the skin of children aged 0 to 12 years with mild to moderate AD compared with those without AD,[60] detecting 19 of 27[61] and 13 of 28[60] of all evaluated inflammatory mediators, respectively. Although a few AD biomarkers, such as helper T cell 2 (T_H2) CCL17 and CCL22, were upregulated in tape strips from AD skin compared with tape strips from normal skin, key AD biomarkers, including those recently reported to be significantly upregulated in pediatric AD skin biopsies compared with normal skin biopsies (IL-4, IL-13, and IL-5),[27,34,35] were either not detected or showed lower protein expression in AD skin.[61]

Two studies performed transcriptomic RNA analyses using Ion AmpliSeq sequencing (ThermoFisher Scientific), primarily focusing on nonlesional skin.[58,73] One study that involved adults with mild to severe AD and adults without AD identified 29 differentially expressed genes.[58] Another study involved children and adolescents aged 8 to 16 years with mild to severe AD, some of whom had food allergies (FAs) and nonatopic dermatitis, reporting that those with AD and FAs had a greater number of barrier- and T_H2-related abnormalities.[73] However, these transcriptomic studies had detection rates of only 18% to 52% for normal skin, 26% to 60% for nonlesional skin, and 57% to 89% for lesional skin (total success rate, approximately 45%), rendering this approach questionable for therapeutic or longitudinal studies.[58,73] A reproducible minimally invasive messenger RNA (mRNA)–based tape-strip profiling approach that defines early-onset pediatric AD biomarkers that can track cutaneous disease in pediatric longitudinal studies or clinical trials is lacking.

Key Points

Question Can tape strips serve as a minimally invasive approach to assess biomarkers for early-onset pediatric atopic dermatitis?

Findings In this cross-sectional study of 51 children younger than 5 years with and without atopic dermatitis, the use of tape strips, a minimally invasive approach for skin sampling, detected the cutaneous immune and barrier abnormalities of early-onset atopic dermatitis in infants and young children and defined biomarkers that are associated with disease severity, pruritus, and transepidermal water loss.

Meaning Minimally invasive tape strips can be used to broadly characterize immune and epidermal barrier biomarkers of the lesional and nonlesional skin of children with early-onset pediatric atopic dermatitis, providing a useful, noninvasive approach for pediatric clinical trials and longitudinal studies.

To evaluate whether tape strips accurately reflected AD activity in skin, we performed mRNA profiling on tape strips from the lesional and nonlesional skin of 21 children who had moderate to severe AD and were less than 6 months from disease initiation and 30 children without AD, all younger than 5 years. We used an expanded panel of 79 genes, including those previously associated with immune and barrier AD abnormalities[8,9,27,33-35,74,75] and those detected in previous AD tape-strip studies.[42,45,47,51-54,56-58,60,63,66,70,71]

Methods

A total of 51 children younger than 5 years were enrolled in the study. Of those, 21 children had new-onset (disease duration <6 months) moderate to severe AD and 30 children did not have AD or a history of personal or family atopy (**Table 1**). Patients were recruited from the dermatology outpatient clinics at the Ann & Robert H. Lurie Children's Hospital of Chicago between January 22, 2016, and April 20, 2018. Patients with active skin infections or who used systemic immunosuppressants within 4 weeks, topical steroids or immunomodulators within 1 week, or moisturizers within 12 hours before evaluation were excluded. Transepidermal water loss (TEWL) was measured, and AD severity was assessed using the SCORing Atopic Dermatitis (SCORAD), Eczema Area and Severity Index (EASI), and Pruritus Atopic Dermatitis Quickscore (ADQ) tools (Table 1). Filaggrin gene (*FLG*) loss-of-function mutations were not evaluated. This study was approved by the institutional review board of the Feinberg School of Medicine, Northwestern University (Chicago, Illinois), and all parents signed written consent forms that were approved by the institutional review board.

Among children with AD, 16 consecutive, large D-Squame tape strips (CuDerm Corp) were collected from the lesional skin of the antecubital fossa (ie, the triangular region in the forearm on the anterior surface of the elbow) when possible. Nonlesional skin was sampled from nearby skin on the same arm. Skin from children without AD was

Table 1. Baseline Demographics and Clinical Characteristics

Characteristic	Children With AD (n = 21)[a]	Children Without AD (n = 30)	*P* Value
Age, mean (SD), y	1.7 (1.7)	1.8 (2.0)	.85
Sex, No. (%)			
Female	6 (28.6)	20 (66.7)	.02
Male	15 (71.4)	10 (33.3)	
Race/ethnicity, No. (%)			
Asian/Pacific Islander	2 (9.5)	6 (20.0)	.30
African American	4 (19.0)	2 (6.7)	
White	15 (71.4)	22 (73.3)	
Clinical severity disease scores, mean (SD)			
SCORAD	54.4 (21.2)	NA	NA
EASI	20.9 (12.6)	NA	NA
TEWL, lesional, g/h/m^2	65.4 (49.2)	NA	NA
TEWL, nonlesional, g/h/m^2	28.1 (18.1)	NA	NA
Pruritus ADQ	16.6 (8.1)	NA	NA
Patient history			
Age at onset of AD, mean (SD), mo	2.3 (1.3)	NA	NA
History of atopy, No. (%)	6 (28.6)	NA	NA
Family history of AD, No. (%)	18 (87.7)	NA	NA

Abbreviations: AD, atopic dermatitis; ADQ, Atopic Dermatitis Quickscore; EASI, Eczema Area and Severity Index; NA, not applicable; SCORAD, SCORing Atopic Dermatitis; TEWL, transepidermal water loss.

[a] Normal value. Mean (SD) TEWL at age 2 months in children without AD has been reported as 10.97 (7.98) g/h/m^2 and at age 6 months has been reported as 10.71 (7.1) g/h/m^2.[76]

sampled from the same areas at the same times. Tape strips were consecutively labeled, and the first 2 tape strips were discarded (eMethods 1 in the Supplement).[52,58,60,63,72,77]

We extracted RNA for quantitative real-time polymerase chain reaction (qRT-PCR) analysis through the miRNeasy Mini Kit (Qiagen), and we used 500 pg total RNA (eMethods 2 in the Supplement). Preamplification was performed on all samples; 1 nonlesional sample was undetectable. TaqMan Low Density Array cards (ThermoFisher Scientific) were used for qRT-PCR, as reported.[78,79] Cycle threshold (Ct) values were normalized to the housekeeping gene *RPLP0* by negatively transforming the Ct values to −dCt. Undetected expression values for each gene were estimated as 20% of the minimum expression across all samples. Primers are listed in eTable 1 in the Supplement.

Immunohistochemistry was performed on frozen tissue sections from children with early pediatric AD (lesional and nonlesional) and children without AD (n = 3 for each group) using purified mouse and rabbit antihuman monoclonal antibodies (eTable 2 in the Supplement).

Statistical Analysis

Statistical analyses were performed using R software, version 3.6.1 (R Foundation for Statistical Analysis), and a linear mixed-effects model was used to analyze $\log_2$ qRT-PCR data. Mean expressions of all markers were summarized in a heat map, in which unsupervised clustering was performed using euclidean distance and average agglomeration criteria. Spearman correlation coefficients were used to evaluate the association between inflammatory markers and disease severity, with $P < .05$ considered significant. To evaluate the performance of an AD classifier, we used the receiver operating characteristic area under the curve (AUC); details are provided in eMethods 3 in the Supplement.

Results

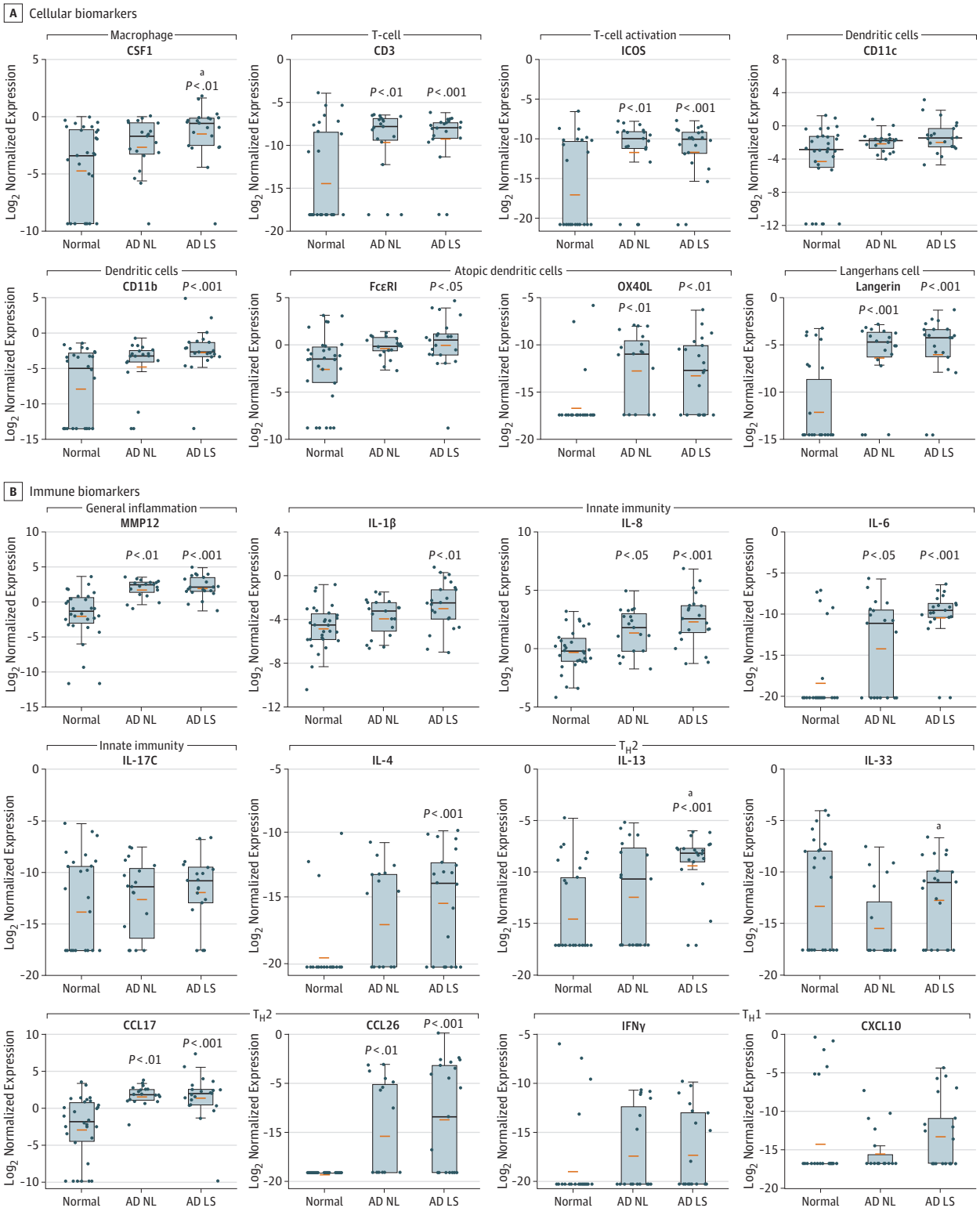
Study Participants

Tape strips were collected from a total of 51 children younger than 5 years; 21 children had moderate to severe AD (SCORAD mean, 54.4) and a disease duration of less than 6 months, and 30 children did not have AD. Of the 21 children with AD, the mean (SD) age was 1.7 (1.7) years, and most were male (15 [71.4%]) and white (15 [71.4%]). Of the 30 children without AD, the mean (SD) age was 1.8 (2.0) years, and most were female (20 [66.7%]) and white (22 [73.3%]). Tape stripping was well tolerated without clinical sequelae. An analysis of a large panel of 79 immune and barrier mediators, including key AD biomarkers[2,21,80,81] in lesional and nonlesional AD skin and normal skin, had a 99% success rate for samples (mRNA was undetectable in only 1 of 71 samples).

Cellular AD Biomarkers

To understand whether tape strips could assess the cellular profile of early AD skin, we analyzed a panel of 15 cellular markers.[58,62,65] We detected markers of monocytes and macrophages,[10,82,83] T cells, activated T_H2 cells, dendritic cells and dendritic-cell subsets,[84,85] and Langerhans cells (langerin protein; **Figure 1** and eFigure 1). Most cellular markers, with the exception of CD83 and CD11c, showed significant differences between lesional AD skin and normal skin. Some markers, such as the OX40 ligand (OX40L) receptor (associated with atopic dendritic cells),[86,87] the inducible T-cell costimulatory activation marker (ICOS), CD209, CD123, and langerin protein, were also significantly increased in nonlesional AD skin (Figure 1; eFigure 1 and eTable 4 in the Supplement).[58,60,61] Only colony-stimulating factor 1 (CSF1) and CSF2 showed significant differences between lesional and nonlesional AD skin.

Figure 1. AD Biomarkers Detected in Tape Strips



A, Cellular biomarkers. B, Immune biomarkers. A set of cellular biomarkers and immune biomarkers were validated and detected using tape strips in normal skin, atopic dermatitis (AD) nonlesional (NL) skin, and AD lesional skin (LS). CSF indicates colony-stimulating factor; ICOS, inducible T-cell costimulator. Boxes depict interquartile range. Whiskers depict the minimum and maximum intervals; the blue dots represent individual samples; orange bars represent means; horizontal black lines represent medians. *P* values denote significance of AD nonlesional or AD lesional versus normal skin.

[a] $P < .05$; significance between AD lesional versus nonlesional skin.

Immune Activation

We next evaluated the mRNA expression of 64 immune and barrier markers previously associated with AD in skin biopsies.[10-12] Many of these markers were below the detection level of microarrays or whole transcriptome shotgun sequencing (RNA-Seq),[10,11,58,61] and many were not detected in previous tape-strip studies (eTable 3 in the Supplement).[53,58,60,61]

The evaluation of immune biomarkers included general inflammation (matrix metallopeptidase 12 [MMP12]), epidermal proliferation, innate immunity, T_H2-associated, T_H1 natural killer T-cell (NKT) activation cytokine, T_H1-associated, T_H17-induced, T_H17/T_H22-associated, regulatory T (Treg) cell, and negative regulator markers (Figure 1 and **Figure 2**; eFigure 1, eTable 2, and eTable 4 in the Supplement). Overall key innate immune (IL-8 and IL-6), T_H2-related (IL-13, IL-4, CCL17, and CCL26), and T_H17/T_H22 (IL-23p19, IL-19, IL-36G, CCL20, β-defensin 4 [DEFB4], cathelicidin LL-37, and S100A proteins [S100As]) measures were significantly upregulated, whereas negative regulators were significantly downregulated across AD vs normal skin. Similar to our recent reports regarding early-onset pediatric AD whole-skin biopsies, and unlike the skin from adults with chronic AD,[27,35] T_H1-associated mRNAs (with the exception of CCL2 and the STAT 1 signaling pathway) were not significantly increased in tape strips from children with early-onset AD (Figure 1; eFigure 1 and eFigure 2 in the Supplement).

Barrier Biomarkers

We also evaluated markers of epidermal differentiation, tight junctions, lipids, and serine proteases. Several epidermal differentiation markers showed significantly reduced expression in both lesional and nonlesional AD tape strips vs normal tape strips. These included FLG, FLG2, loricrin (LOR), periplakin (PPL), and psoriasis susceptibility 1 candidate 2 (PSORS1C2; Figure 2 and eTable 4 in the Supplement).

As in our early-onset pediatric AD biopsies,[27,28] tape-strip mRNA expression of some lipid (fatty acid 2-hydroxylase [FA2H] and fatty acyl-CoA reductase 2 [FAR2]) and tight junction (CLDN8 and CLDN23) products was downregulated in both lesional and nonlesional AD skin compared with normal skin (Figure 2 and eFigure 2 in the Supplement). Other lipid markers showed no differential expression in tape strips (eFigure 2). The serine proteases KLK5 and KLK7 were significantly downregulated in AD vs normal skin (Figure 2; eTable 4 and eFigure 2 in the Supplement). Only 2 markers (CCL11 and ELOVL fatty acid elongase 3 [ELOVL3]) were undetectable.

A summary heat map of all 79 immune and barrier measures depicts mRNA expression differences (as fold changes) among lesional and nonlesional AD tape-stripped skin samples compared with normal tape-stripped skin samples (**Figure 3** and eTable 4 in the Supplement). Significantly downregulated products in AD skin include barrier measures (ie, LOR, PPL, FLG, CLDN23, and FA2H) and negative regulators (IL-34 and IL-37). Markers significantly upregulated in AD skin include multiple immune genes representing dendritic cells (CD11b [ITGAM] and OX40L), T-cell activation (ICOS), T_H2 (CCL17, IL-4, and IL-13), and T_H17/T_H22 (IL-19, IL-23p19, IL36G, S100As, and CCL20) (Figure 3). Means, *P* values, SEs, and CIs are summarized in eTable 4 in the Supplement.

Because older children and adolescents with AD and FAs were reported to have differences in tape strips compared with those with AD and no FAs, we conducted a sensitivity subanalysis of 6 children with FAs compared with 15 children without FAs to evaluate whether the coexistence of atopic comorbidities was associated with changes in the skin phenotype. Data were similar between children with AD with and without other atopic manifestations. A sensitivity subanalysis evaluating sex as a confounder found no sex-related differences.

Protein Validation of Selected Mediators

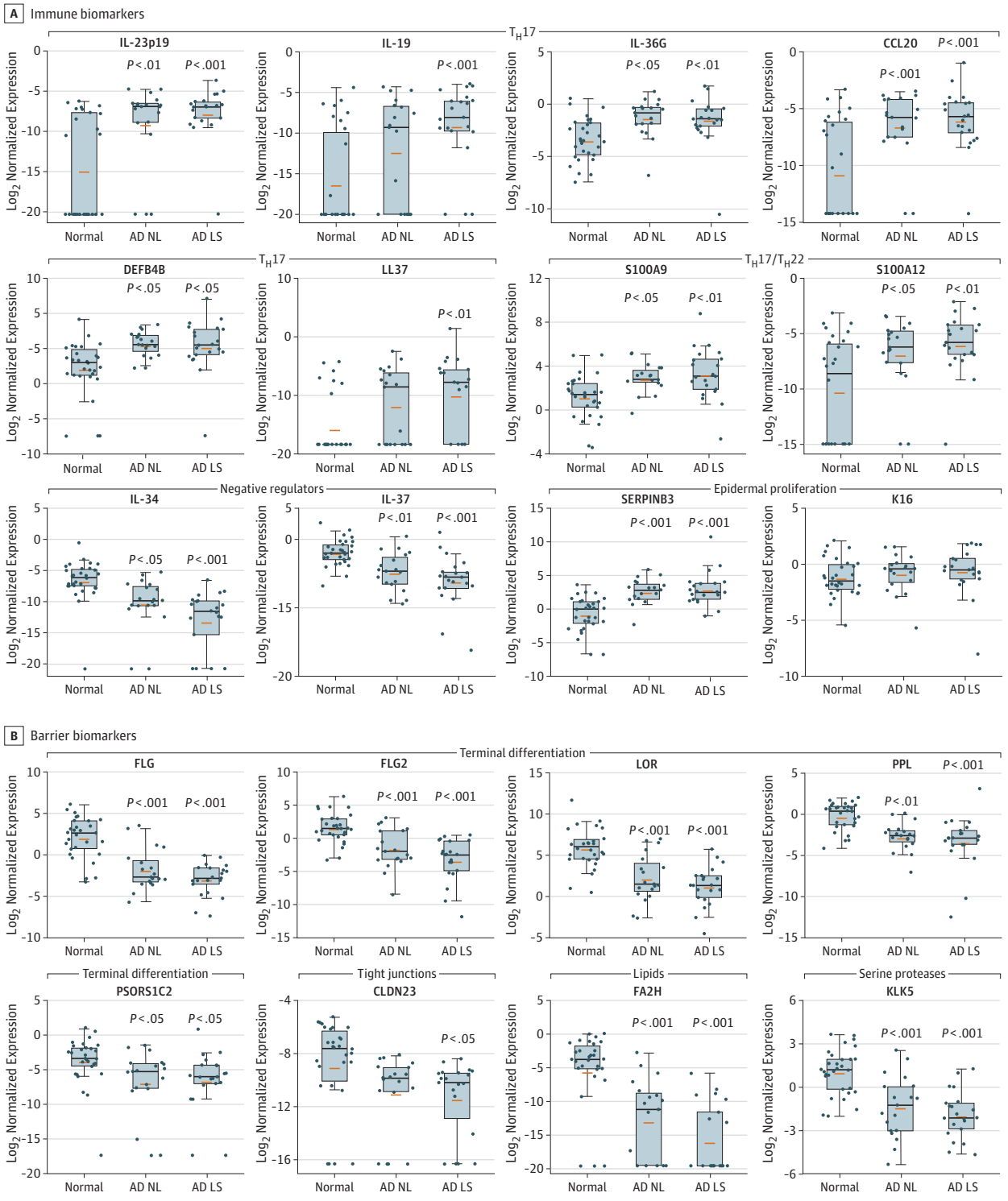
We further validated the protein expression of dendritic cell (CD11b) and epithelial cytokine (IL-33 and IL-17C) markers in tissue sections from children aged 0 to 5 years with moderate to severe pediatric AD and those without AD using immunohistochemistry.[27,28] Although most $CD11b^+$ dendritic cells are located in the dermis, we detected foci of cellular infiltrates in the outer epidermis, primarily in AD lesions (eFigure 3 in the Supplement). Immunostaining for epidermal cytokines (IL-33 and IL-17C)[28,88,89] showed diffuse, more intense epidermal staining in both lesional and nonlesional AD skin, with fainter staining that was more localized to the lower epidermis in normal skin (n = 3 for all tissues; eFigure 3 in the Supplement).

Tape-Strip Biomarkers and Clinical Disease

To determine how molecular and cellular tape-strip biomarkers from early-onset pediatric AD lesional and nonlesional skin correlate with clinical severity (based on SCORAD, EASI, and Pruritus ADQ assessments) and epidermal barrier function (based on TEWL assessment), we performed a Spearman correlation coefficient analysis (**Table 2** and eFigure 4 in the Supplement). The SCORAD assessment showed the greatest number of significant correlations with lesional biomarkers. Significant correlations with SCORAD and EASI assessments were noted with the expression of lesional T_H2 (IL-33) and T_H17 (IL-23p19) cytokines (Table 2 and eTable 4 in the Supplement). Key T_H17/T_H22 (IL-19, S100As, PI3, IL-36G, β-defensin 4B [DEFB4B], STAT 3, and cathelicidin LL-37; eFigure 4 in the Supplement), innate (IL-17C), hyperplasia (epidermal proliferation marker K16), T_H2 (IL-4R), and cellular (CD11b and CD11c) biomarkers in lesional skin were also significantly associated with disease severity (Table 2 and eTable 4 in the Supplement). Significant correlations were found between pruritus and lesional AD markers (cellular markers: CD209, CD11b, CSF2, and CD3; T_H2-associated markers: CCR4, CCL18, IL-10, and IL-13; and T_H1-associated markers: immune interferon [IFNγ] and chemokines CXCL9 and CXCL11). Transepidermal water loss was positively associated with several immune markers (T_H1, IFNγ, T_H17/T_H22, IL-19, S100A7, T_H2, and CCL26) and negatively associated with LOR (Table 2).

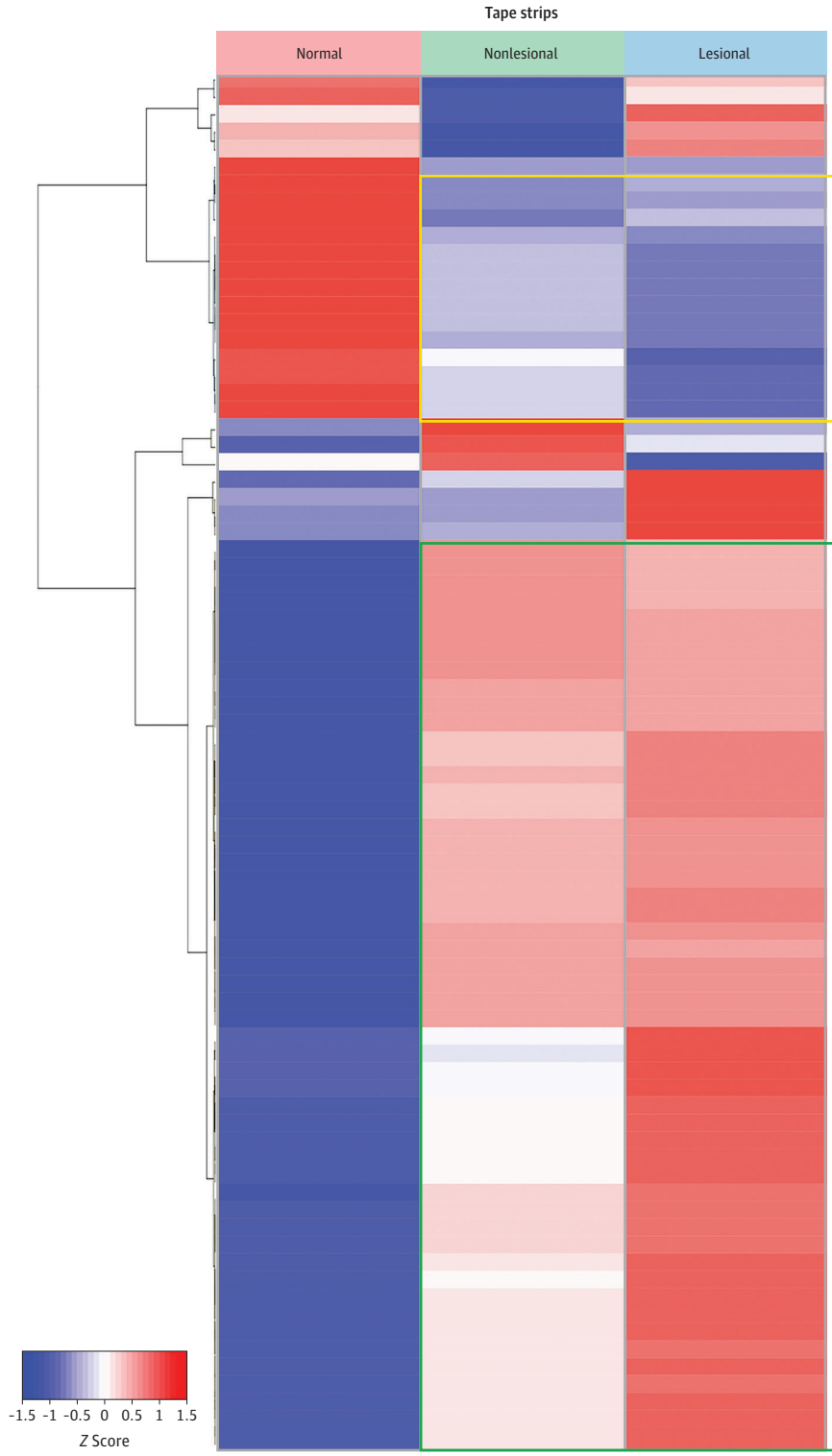
Both SCORAD and EASI measurements were correlated with biomarkers in nonlesional AD skin, including innate (IL-17C), T_H17/T_H22 (IL-23p19, IL-36G, DEFB4B, phosphatidylinositol 3 [PI3], and S100A12; eFigure 4 in the Supplement), and lipid (FAR2) measures (Table 2 and eTable 4 in the Supplement). Additional T_H17/T_H22 (IL-19, S100A8, and S100A9) and epidermal hyperplasia/proliferation (K16 and serine protease inhibitor B3 [SERPINB3]) biomarkers were positively correlated with EASI

Figure 2. Key Immune and Barrier AD Biomarkers Detected in Tape Strips



A, Immune biomarkers. Epidermis-derived barrier biomarkers show differential expression across atopic dermatitis (AD) skin vs normal skin. B, Barrier biomarkers. Immune biomarkers show greater differential expression across AD skin vs normal skin in tape strips in normal skin, AD nonlesional (NL) skin, and AD lesional skin (LS). Boxes depict interquartile range. Whiskers depict the minimum and maximum intervals; the blue dots represent individual samples; orange bars represent means; horizontal black lines represent medians. *P* values denote significance of AD nonlesional or AD lesional versus normal skin.

Figure 3. Heat Map of Immune and Barrier Atopic Dermatitis (AD) Biomarkers Detected in Tape Strips



Biomarker	NL vs N	LS vs N	LS vs NL
CXCL11	-2.17	-1.25	1.74
ELOVL3	-2.70	-1.54	1.75
CXCL10	-2.25	1.89	4.24
CXCL9	-2.76	1.14	3.14
IL33	-4.93	1.52	7.51[a]
CDSN	-3.50	-3.20	1.09
PSORS1C2	-9.87[a]	-6.96[a]	1.42
FAR2	-2.28	-1.89	1.21
CLDN8	-5.61	-3.94	1.43
SCD	-2.65	-2.84	-1.07
LOR	-12.90[c]	-25.40[c]	-1.97
KLK7	-2.34	-2.80[a]	-1.20
FLG	-14.80[c]	-33.50[c]	-2.26
KLK5	-5.44[c]	-7.97[c]	-1.47
PPL	-5.55[b]	-8.35[c]	-1.50
CLDN23	-4.53	-5.47[a]	-1.21
IL34	-14.20[a]	-89.40[c]	-6.28
FLG2	-10.60[b]	-26.90[c]	-2.53
IL37	-9.07[b]	-17.80[c]	-1.96
FA2H	-178.00[c]	-1450.00[c]	-8.16
CCL22	1.48	1.08	-1.37
CD8E	1.21	1.22	1.00
CERS3	1.19	-1.37	-1.64
CCL3	1.13	3.15	2.79
DGAT2	-1.06	1.76	1.86
MX1	-1.04	3.93	4.10
IL1γ	-1.01	1.96	1.98
OX40L	14.90[b]	10.70[b]	-1.40
IL2	7.52[b]	5.74[b]	-1.31
S100A7	3.64[a]	3.45[a]	-1.06
IL36G	3.49[a]	3.84[b]	1.10
CCL17	16.30[b]	18.60[c]	1.15
DEFB4B	7.86[a]	9.07[a]	1.15
CLDN1	4.96	5.05[a]	1.02
IL4R	1.99	2.32	1.16
IL3RA	5.49[a]	6.59[b]	1.20
CD28	3.83	4.08[a]	1.07
CCL18	18.50	19.00[a]	1.03
ELOVL5	2.95	5.56	1.89
S100A12	8.71[a]	18.70[b]	2.15
S100A9	3.13[a]	4.29[b]	1.37
IL2RA	7.93	19.20[b]	2.42
IL23p19	41.60[b]	136.00[c]	3.28
ITGAX	3.36	4.94	1.47
SERPINB3	10.00[c]	13.40[c]	1.33
CD3	25.80[b]	39.20[c]	1.52
CCL20	15.20[b]	26.80[c]	1.76
FCεRI	3.75	5.96[a]	1.59
IL10	17.10[c]	27.70[c]	1.62
S100A8	2.92	3.85[a]	1.32
ICOS	37.60[b]	44.70[c]	1.19
CCR4	6.97	9.46[a]	1.36
MMP12	13.50[b]	16.00[c]	1.18
CD207	52.40[c]	73.50[c]	1.40
IFNγ	3.19	3.19	1.00
CXCL1	2.67	13.60[a]	5.07
IL13	3.94	35.20[c]	8.93[a]
IL1RA	1.84	3.47[c]	1.88
IL1β	1.96	3.71[b]	1.89
STAT3	1.57	2.67	1.70
CSF2	7.12	63.10[c]	8.87[a]
IL19	14.00	143.00[c]	10.20
CCL4	2.54	7.54	2.97
IL6	18.20[a]	234.00[c]	12.90
CCL26	15.60[b]	50.10[c]	3.22
LL37	13.50	52.00[b]	3.85
FOXP3	5.31	13.80[b]	2.60
CCL11	1.28	1.43	1.12
CCL2	5.17	16.30[b]	3.16
STAT1	2.19	4.57[a]	2.09
IL12/23p40	5.42	19.90[b]	3.67
ITGAM	7.72	37.40[c]	4.85
IL4	5.98	19.20[c]	3.21
PI3	1.25	1.86	1.48
K16	1.14	1.50	1.32
IL8	3.13[a]	6.30[c]	2.01
CSF1	3.40	9.32[b]	2.74[a]
CD209	6.82[a]	22.10[c]	3.25
IL17C	2.07	3.75	1.81

Mean expression levels of all 79 barrier and inflammatory mediators detected using tape strips (measured by real-time polymerase chain reaction). The table shows biomarkers with signed fold changes in nonlesional (NL) AD skin vs normal (N) skin, lesional (LS) AD skin vs normal skin, and LS AD skin vs NL AD skin. The yellow box indicates the significant downregulation of biomarkers in LS and NL AD skin compared with normal skin; the green box indicates the significant upregulation of biomarkers in LS and NL AD skin compared with normal skin.

[a] $P < .05$.

[b] $P < .01$.

[c] $P < .001$.

Table 2. Spearman Correlations of Atopic Dermatitis (AD) Biomarkers

	Assessment Tool											
	SCORAD			EASI			Pruritus ADQ			TEWL		
Correlation Rank	Marker	ρ	*P* Value	Marker	ρ	*P* Value	Marker	ρ	*P* Value	Marker	ρ	*P* Value
Correlations With Clinical Indices of AD in Pediatric Lesional Skin Using Tape Strips												
1	EASI	0.784	<.001	SCORAD	0.784	<.001	CD209	0.814	<.001	IFNγ	0.597	.02
2	SERPINB3	0.676	.003	IL-33	0.498	.04	CCR4	0.670	.01	IL-19	0.570	.03
3	IL-33	0.651	.005	IL-23p19	0.494	.04	LL37	0.635	.02	S100A7	0.565	.04
4	IL-36G	0.608	.01	Pruritus ADQ	0.482	.08	CD11b	0.579	.03	Pruritus ADQ	0.564	.10
5	CD11b	0.594	.01	CCR4	0.475	.047	CCL18	0.572	.03	ELOVL3	0.563	.04
6	IL-4R	0.594	.01	K16	0.455	.06	IL-10	0.570	.03	IL-33	0.469	.09
7	S100A9	0.591	.01				IFNγ	0.564	.04	CCL26	0.453	.10
8	S100A8	0.578	.02				DEFB4B	0.552	.04	FLG	−0.495	.08
9	DEFB4B	0.571	.02				CSF2	0.542	.045	LOR	−0.543	.048
10	PI3	0.564	.02				CXCL9	0.531	.05			
11	K16	0.564	.02				CXCL11	0.530	.05			
12	IL-23p19	0.548	.02				CD3	0.526	.05			
13	STAT3	0.531	.03				IL-13	0.524	.05			
14	ELOVL5	0.531	.03	NA	NA	NA						
15	S100A12	0.527	.03							NA	NA	NA
16	IL-17C	0.521	.03									
17	KLK7	0.513	.04									
18	CD11c	0.484	.049									
19	LL37	0.478	.05									
20	IL-19	0.473	.06									
Correlations With Clinical Indices of AD in Pediatric Nonlesional Skin Using Tape Strips												
1	EASI	0.774	.001	SCORAD	0.774	.001	CXCL9	0.797	.002	DGAT2	0.629	.03
2	IL-23p19	0.643	.01	IL-17C	0.642	.01	IL-19	0.709	.01	S100A7	0.622	.04
3	FAR2	0.633	.01	PI3	0.632	.01	CXCL10	0.616	.03	SERPINB3	0.552	.07
4	S100A12	0.626	.01	DEFB4B	0.629	.01	IL-12/IL-23p40	0.586	.045	IL-4R	0.545	.07
5	IL-17C	0.597	.02	S100A9	0.609	.01	EASI	0.567	.05	S100A8	0.524	.08
6	IL-36G	0.584	.02	IL-36G	0.606	.02	IL-2	0.555	.06	K16	0.524	.08
7	PI3	0.579	.02	SERPINB3	0.594	.02	PI3	0.550	.06	IL-1RA	0.524	.08
8	DEFB4B	0.552	.03	Pruritus ADQ	0.567	.06	LL37	0.519	.08	CD11c	0.517	.09
9	IL-19	0.471	.08	K16	0.556	.03	S100A9	0.511	.09	FcεRI	0.503	.10
10	SERPINB3	0.427	.11	FAR2	0.550	.03	IL-37	−0.494	.10	SCD	−0.609	.04
11				S100A12	0.550	.03	FLG2	−0.529	.08			
12				S100A8	0.526	.04	CCL22	−0.539	.07			
13	NA	NA	NA	IL-19	0.513	.04	FLG	−0.620	.03	NA	NA	NA
14				S100A7	0.500	.05	LOR	−0.683	.01			
15				IL-23p19	0.487	.06	NA	NA	NA			

Abbreviations: ADQ, Atopic Dermatitis Quickscore; EASI, Eczema Area and Severity Index; SCORAD, SCORing Atopic Dermatitis; TEWL, transepidermal water loss; NA, not applicable.

measurements (eTable 4 in the Supplement). Pruritus ADQ showed significant positive correlations with T_H1 NKT-associated (CXCL9 and CXCL10), and T_H17-related (IL-12, IL-23p40, and IL-19) mediators, and significant negative correlations with differentiation markers (FLG and LOR). Transepidermal water loss was significantly positively associated with T_H17/T_H22, S100A7, and diglyceride acyltransferase 2 (DGAT2; Table 2).

Finally, we evaluated whether any profiled biomarker in tape strips could accurately discriminate between pediatric AD skin and normal skin. The epidermal negative regulator cytokine IL-34 was the best single-gene classifier, discriminating AD from normal skin with almost 100% accuracy (AUC, 0.94). Epidermal barrier markers (FLG, FLG2, LOR, and FA2H) were also effective discriminators (AUC, 0.93-0.90; eFigure 5 in the Supplement). Integration of the top differentially expressed immune and barrier markers (defined as markers with AUC values greater than 0.65) resulted in a combined score that discriminated AD skin from normal skin with nearly 100% accuracy (AUC, 1). The predictor included key AD biomarkers that contributed positively (CSF1, MMP12, CCL26, CCL20, IL-13,

S100A7, and S100A9) as well as negatively (IL-34 and barrier genes, *FLG*, *FLG2*, *LOR*, *PSORS1C2*, *FA2H*, and *FAR2*; eFigure 5 in the Supplement) to the score. Only lesional biomarkers were able to discriminate between AD and normal skin.

Discussion

To our knowledge, this is the first study to provide a broad characterization of immune and barrier abnormalities in the skin of children with early-onset (aged <5 years) AD skin using a minimally invasive tape-strip approach. Because tissue biopsies are considered the criterion standard for evaluating dysregulation in AD lesional and nonlesional skin, [2,6-10,12,16-18,20,27-29,78,86,90-92] it is crucial to understand whether tape-strip profiling can accurately yield key AD-related biomarkers. While this minimally invasive approach can be useful across AD endotypes,[8,9,14,21,27-29,58,78,90] it is particularly important for studying pediatric AD skin owing to the impracticality of performing biopsies in children. By defining a minimally invasive method for biomarker detection in early-onset pediatric AD skin, we can potentially help evaluate disease progression in longitudinal studies and monitor early disease reversal in clinical trials.[6,18,19]

Most previous tape-strip studies primarily focused on protein profiling in the skin of adults with chronic AD (eTable 3 in the Supplement)[42,53,54,57,58,69] and evaluated a limited panel of inflammatory and/or barrier-related proteins. Two studies analyzed protein expression in tape strips from children with AD.[60,61] While these studies used nearly identical methods to measure protein expression, their results and conclusions were largely dissimilar. Hulshof et al[75] detected 13 of the 28 assessed immune markers in tape strips from the lesional AD, nonlesional AD, and normal skin of children aged 0 to 12 years, with no significant increases in nonlesional AD skin compared with normal skin and with significant downregulation in 3 markers (IL-1α, CCL4, and CXCL10). Seven cytokines (eg, IL-1β, CCL17, CCL18, and IL-8) were significantly increased in lesional vs nonlesional and normal skin.

In contrast, McAleer et al[61] studied only the nonlesional skin of infants with early-onset AD vs infants without AD, detecting 19 of the 27 analyzed inflammatory products, with significant increases in 11 cytokines in nonlesional AD vs normal tape-stripped skin. The study also detected significantly decreased expression of key T_H2 and T_H17 cytokines (IL-13, IL-5, IL-12, IL-23p40, and CSF2).[45] Some key T_H2 (IL-4, CCL13, and CCL26), T_H1 (IFNγ), and other cytokines could not be detected by these studies. Significant correlations between AD severity (based on SCORAD assessment) with CCL17 and IL-8 expression in nonlesional and/or lesional skin were reported ($r = 0.4$; $P < .05$ for both comparisons).[60,61] Two recent studies evaluated RNA profiling using Ion AmpliSeq sequencing (ThermoFisher Scientific) to analyze tapes 11 to 20.[58,73] One study focused on gene expression in adults with nonlesional mild to severe AD vs adults without AD[58] and detected 29 differentially expressed genes (false discovery rate, <0.05),[58] including several immune genes (*HLA-DOB*, *S100A7A*, *MMP9*, *MMP10*, and *CXCL6*). A subset of 9 patients with higher inflammation and disease severity was identified, with upregulation of T_H2 products (IL-13, IL-4R, CCL22, and CCR4). Detection rates for nonlesional AD and normal skin were 60% and 52%, respectively. Another recent study[73] evaluated primarily nonlesional skin from children and adolescents aged 8 to 16 years with mild to severe AD, focusing on those with and without concurrent FAs (n = 21 and n = 19, respectively) vs children and adolescents without AD (n = 22). The overall success rate for transcriptome sequencing was 45% of all samples (18% for normal skin, <40% for nonlesional skin, and 57%-89% for lesional skin detection). The study reported higher expression of T_H2 products (IL-4R and thymic stromal lymphopoietin [TSLP] receptor) and lower expression of FLG breakdown products and lipids in the nonlesional skin of children and adolescents with AD and FAs compared with those with AD and no FAs and those without AD.[73]

This study expands on previous profiling studies,[42,45,53,54,57,58,66,68,69,93] including 2 RNA-Seq studies that reported a 45% overall detection rate.[58,73] This study also identifies the largest set of differentially expressed products between AD (both lesional and nonlesional) and normal tape-stripped skin, with a 99% sample detection rate (only 1 of 71 samples was undetectable), which is critical for paired sample analysis. Given our high sample detection rate, it is probable that 2 sequential samples in clinical trials or longitudinal studies will both generate measures.[10-12,27,28,51,53,54,57,58,60,61,69,72,78,84,94,95] Seventy-seven of 79 evaluated immune and barrier gene products were detected (gene detection rate, 97%) from 70 of 71 tape-strip samples (sample detection rate, 99%), with 53 of 79 markers significantly different between children with lesional and/or nonlesional AD and children without AD. Among these mediators were key AD biomarkers, many of which were not detected or evaluated in previous AD tape-strip studies.[53,54,58,60,61] These biomarkers included measures of cellular infiltrates, including those associated with T cells and T-cell activation (CD3 and ICOS), atopic dendritic cells (Fc ε RI and OX40L),[84,86] and key inflammatory markers of general inflammation (MMP12), innate immunity (IL-8 and IL-1RA), T_H2 (IL-4, IL-13, CCL17, and CCL26), T_H17/T_H22 (IL-19, IL-23p19, IL-36G, CCL20, S100As, and DEFB4), Treg cells (FOXP3 and IL-10), negative regulators (IL-34 and IL-37), and epidermal proliferation (SERPINB3). The novel epidermal cytokines IL-33 and IL-17C, which are currently targeted in clinical trials of patients with AD, were also highlighted as novel tape-strip biomarkers[88,96] and demonstrated significant correlations with AD severity. Interestingly, CCL20 has also been reported to be associated with commensal microbes in early life that induce Treg cells,[97] which had increased markers in our study.

Terminal differentiation mRNAs (FLG, FLG2, LOR, and PPL) had decreased expression in lesional and nonlesional early-onset AD tape-stripped vs normal tape-stripped skin, similar to whole biopsies from chronic adult AD skin[10,11,27,28,84] and in contrast with the normal expression of terminal differentiation products in our recent biopsy studies of early-onset pediatric AD.[27,28] Lipid (FA2H) and tight junction (CLDN8 and CLDN23) mRNAs showed similar decreases in early-onset AD

tape strips and biopsies.[27,28] Terminal differentiation measures were generally not reduced in adult AD tape-strip genomic and proteomic studies.[54,58] Few lipid and FLG degradation products showed lower expressions in children and adolescents with AD and FAs compared with those with AD and no FAs and those without AD. The effect of the epidermal depth on expression patterns in serial tape strips deserves further study.[40,52,53,57,61]

Our tape-strip data support the view that the nonlesional skin of patients with early-onset AD is widely abnormal. The significant correlations between the expression of AD-associated biomarkers (T_H2, IL-33, IL-4R, CCR4, and CCL18) in nonlesional skin and severity indices (based on SCORAD, EASI, Pruritus ADQ, and TEWL assessments) further suggest that nonlesional abnormalities may be directed by the extent of active disease. This tape-strip method may be useful, together with TEWL assessment, for early studies of children who are at high risk of developing AD to dissect the sequence of epidermal vs immune changes leading to AD initiation.

Tape strips provide a perfect classifier (defined as an AUC score of 1, with 0 indicating a classifier with no power) to discriminate between early-onset AD skin and normal pediatric skin, including biomarkers pathogenically linked to AD (ie, IL-13, CCL26, S100As, and FLG) and an almost perfect molecular classifier using the negative regulator cytokine IL-34.[10,98] This classifier may be useful in future longitudinal studies that evaluate the resolution of AD. Interleukin 34, a primarily epidermal cytokine and negative regulator of inflammation,[98] has been recently suggested as a treatment-response biomarker in the skin of adults with AD.[6]

Limitations

The study had several limitations, primarily related to tape stripping. First, it is unclear whether tape strips in AD vs normal skin attain similar epidermal depth, especially given the greater mRNA yield in AD skin. Thus, differential expression of some genes may have merely reflected a greater depth of tape stripping within the stratum corneum.[57,58,73] Second, mRNA extraction from tape strips is much more labor intensive compared with biopsies, potentially limiting widespread use. Third, some AD biomarkers, such as the hyperplasia-related K16, which is characteristically upregulated in AD skin,[2,12,20,27,28] were not upregulated in early-onset AD tape strips, although K16 tape-strip mRNA remained correlated with AD severity. Tape strips allow detection of molecular abnormalities in superficial keratinocytes, whereas most K16 induction takes place in basal and suprabasal keratinocytes. Fourth, some AD biomarkers, such as IL-22, are restricted to the dermis[9,10,84,95] and cannot be detected using tape strips. Fifth, our study did not have a direct comparison with whole skin biopsy biomarkers from the same patients. We plan to determine the correlation between skin biopsy and tape-stripping findings in future studies. Sixth, mRNA contained in corneocytes may be less stable than the protein products it encodes; thus, protein staining may show more precise differences, as reported in this article.

Conclusions

Through the use of a minimally invasive tape-strip method, we reported extensive gene-profiling characterization of early-onset moderate to severe pediatric AD skin. This characterization identified the molecular measures of disease activity that precede the onset of clinically visible lesions and may be useful for identifying biomarkers associated with AD. Because tape stripping is painless, nonscarring, and allows repeated sampling, it may be associated with benefits for longitudinal pediatric studies and clinical trials, in which serial measures are needed to identify predictors of response, course, and comorbidities.

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