Transcriptomic Profiling of Tape-Strips From Moderate to Severe Atopic Dermatitis Patients Treated With Dupilumab

Daniela Mikhaylov, BA,* Ester Del Duca, MD,† Caroline Meyer Olesen, MD,‡ Helen He, MD,*, Jianni Wu, BS,*,§ Benjamin Ungar, MD,*, Yeriel Estrada, BS,*, Ning Zhang, MD,*, Mashkura Chowdhury, MS,§ Maja-Lisa Clausen, MD, PhD,† James G. Krueger, MD, PhD,¶ Ana B. Pavel, PhD,¶ Tove Agner, MD, DMSc,¶ and Emma Guttman-Yassky, MD, PhD*

Background: Tape-strips are a minimally invasive approach to characterize skin biomarkers in atopic dermatitis (AD). However, they have not yet been used for tracking gene expression changes with systemic treatment.

Objective: The aim of the study was to evaluate gene expression changes and therapeutic response biomarkers in AD patients before and after dupilumab (interleukin 4Rα antibody) treatment using tape-strips to obtain epidermal tissue for analysis.

Methods: Lesional and nonlesional tape-stripped skin was sampled from 18 AD patients before and after dupilumab treatment and from 17 healthy subjects and analyzed by RNA-seq.

Results: At baseline, we detected 6745 and 4859 differentially expressed genes between lesional and nonlesional skin versus normal, respectively, whereas 841 and 977 genes were differentially expressed after treatment, respectively (fold change >1.5 and false discovery rate <0.05). Tape-strips captured significant modulation with dupilumab in key AD immune (eg, C-C motif chemokine ligand 13 [CCL13], CCL17, CCL18) and barrier (eg, periplakin, FA2H) biomarkers. Changes in biomarkers (CCL20, interleukin 34, FABP7) were also significantly correlated with clinical disease improvements (Eczema Area and Severity Index; R > 0.5 or R < −0.4, P < 0.05).

Conclusions: This real-life study represents the first comprehensive RNA-seq molecular profiling of tape-strips from moderate to severe AD patients after dupilumab therapy. Analysis of tape strip specimens detected significant gene expression changes in key AD biomarkers with dupilumab treatment, suggesting that this approach may be useful to monitor therapeutic responses in inflammatory skin diseases.

ABBREVIATIONS: AD = atopic dermatitis, CCL = C-C motif chemokine ligand, CXCL = C-X-C motif chemokine ligand, DC = dendritic cell, EASI = Eczema Area and Severity Index, FCH = fold change, FDR = false discovery rate, GSVA = gene set variation analysis, IL = interleukin, PPL = periplakin, T1H = T helper

Atopic dermatitis (AD) is one of the most common inflammatory skin diseases, affecting 3% to 10% of adults worldwide.1–3 The pathogenesis of AD is multifactorial and involves infiltration of activated T cells and dendritic cells (DCs),4–7 prominent T helper 2 (T1H2)/T1H22 activation with variable T1H1/T1H17 contributions,8–15 and alterations to the epidermal barrier, including impaired terminal

A Minireview

From the *Department of Dermatology, and Laboratory of Inflammatory Skin Diseases, Icahn School of Medicine at Mount Sinai, New York; †Department of Dermatology, University of Magna Graecia, Catanzaro, Italy; ‡Department of Dermatology, Bispebjerg Hospital, University of Copenhagen, Denmark; ¶State University of New York Downstate Medical Center, College of Medicine, Brooklyn; §The Laboratory for Investigative Dermatology, The Rockefeller University Hospital, New York; and ¶Department of Biomedical Engineering, The University of Mississippi, University, Mississippi.

Address reprint requests to Emma Guttman-Yassky, MD, PhD, Department of Dermatology and the Laboratory of Inflammatory Skin Diseases, Icahn School of Medicine at Mount Sinai Medical Center, 5 E 98th St, New York, NY 10029. E-mail: emma.guttman@mountsinai.org.

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The institutional review board approval status was reviewed and approved by local institutional review board. Informed consent was obtained from all subjects.

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dupilumab, a monoclonal antibody against interleukin (IL) 4RA that inhibits T_{H2} signaling via IL-4/IL-13, is effective in reducing clinical symptoms and molecular inflammation in AD patients with moderate to severe disease.\cite{20, 21, 42} The available molecular studies that assess molecular changes in skin of patients treated with dupilumab have primarily relied on skin biopsies, which are associated with pain, scarring, and cutaneous infections.\cite{23}

Tape stripping is a minimally invasive approach used to sequentially sample the stratum corneum and upper stratum granulosum.\cite{24, 25} Several studies used RNA and protein profiling to characterize skin samples collected by tape-strips from pediatric and adult AD patients.\cite{26, 27, 38, 40} Particularly, broad profiling using RNA-seq allowed identification of immune and barrier abnormalities characterizing AD skin.\cite{38, 40} Recently, tape-strips have been used to identify RNA biomarkers of therapeutic response to topical treatments.\cite{27, 41} Thus far, gene expression studies have been performed in full-thickness skin biopsies to identify biomarkers of therapeutic response in AD clinical trials.\cite{8, 20, 21, 42, 47} To date, gene expression tape-strip studies in moderate to severe AD patients treated with highly effective systemic medications, such as dupilumab, are not available.

The current study represents the first comprehensive RNA-seq molecular profiling of tape-strips from moderate to severe AD patients before and after 16 weeks of dupilumab treatment in a real-life setting. We studied lesional and nonlesional tape-stripped skin of 18 AD patients and 17 healthy controls, accurately capturing immune and barrier changes with dupilumab treatment similar to prior biopsy studies.\cite{27, 48} These results suggest that tape stripping may provide a useful minimally invasive approach for monitoring changes with treatment in the AD cutaneous signature, with numerous clinical applications, including for children.

**METHODS**

**Study Population and Characteristics**

Eighteen White adults with moderate to severe AD (3 female/15 male adults, mean age = 43.6 years, pretreatment mean Eczema Assessment Severity Index (EASI) = 20.7, posttreatment mean EASI = 5.6) were enrolled from the Department of Dermatology, Bispebjerg Hospital, Denmark, between March 2018 and June 2019 (Table 1), and 17 healthy volunteers (10 female/7 male volunteers, mean age = 39.3 years) were enrolled at the Department of Dermatology at Mount Sinai, New York, under institutional review board–approved protocols. Informed consent was obtained from all subjects. No significant demographic differences were observed between groups besides differences in sex distribution. A sensitivity analysis that adjusted for sex did not significantly change results (data not shown). All AD patients were treated with a loading dose of dupilumab 600 mg, followed by every-other-week dupilumab 300 mg (label use) for a total of 16 weeks. Patients had to meet the following criteria to be eligible for dupilumab treatment: AD according to the UK criteria, age of 18 years or older, EASI score of greater than 16, DLQI of greater than 10, and failure on previous treatment with 2 or more traditional systemic immunosuppressive therapies. Patients transferred to dupilumab from systemic immunosuppressive therapy could receive dupilumab despite EASI of less than 16. Patients were instructed not to apply emollients within 24 hours of each visit and not to use topical anti-inflammatory treatments 7 days before each evaluation. All AD patients reported no known history of other inflammatory or autoimmune diseases (eg, psoriasis, rheumatoid arthritis).

**Tape-Strip Collection**

Tape-strips were collected from AD patients before and after 16 weeks of dupilumab therapy and from healthy controls at the initial visit. For each sample, 30 serially labeled tape-strips (D-Squame 3.8 cm²; CuDerm) were collected from the upper or lower extremities. Every tape was pressed down on the skin for 10 seconds with a standardized pressure (225 g/cm²), using the D-Squame pressurizer. Lesional and nonlesional skin was sampled from the same extremity but at least 10 cm apart. Tape-strips were collected before and after dupilumab treatment at adjacent sites from the same lesions to avoid any bias in sampling. Disease severity was assessed with the Eczema and Severity Index (EASI).\cite{49}

The collection of tape-strips across both sites used the same methods and pressurized device to standardize the collection. All the laboratory analyses were done in one laboratory, at Mount Sinai.

**RNA Extraction and RNA-Seq**

RNA was extracted from tape-strips using miRNAeasy Mini Kit (Qiagen, Hilden, Germany). The RNA yield across tape-strip samples was 31.11 ± 55.82 ng (mean ± SD). RNA AmpliSeq libraries were constructed with the Ion AmpliSeq Transcriptome Human Gene Expression Kit, using an input of 5 ng RNA per sample. This method uses a multiplexed amplification approach that screens more than 20,000 genes per reaction. RNA-seq libraries were pooled and sequenced on the Ion Torrent Proton sequencer with P1 chips. RNA was extracted, and RNA-seq was performed on all samples.

**Statistical Analysis**

Statistical analyses were performed using R software (http://www.R-project.org) and packages available through Bioconductor (http://www.bioconductor.org), as previously described.\cite{30, 35} Sample quality was assessed with FastQC. Samples were aligned to the human reference genome using TopHat2 and then mapped to the human genome (hg19) with Bowtie2. RNA-seq alignments were processed with StringTie and assembled using a standard splice-junction gtf file. Gene expression data were normalized with edgeR. The library sizes were normalized with DESeq2. The data were log transformed, and a negative binomial distribution was modeled for each condition. Differential expression analysis was performed with edgeR. To control for multiple testing, the false discovery rate was adjusted in the DESeq2 output using the `qvalue` package. The analysis was performed using an adjusted p-value cutoff of 0.05 (FDR ≤ 0.05).

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### Table 1: Demographic Table

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Healthy (n = 17)</th>
<th>Atopic Dermatitis (n = 18)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, mean ± SD, y</td>
<td>39.3 ± 14.6</td>
<td>43.6 ± 10.1</td>
<td>0.320</td>
</tr>
<tr>
<td>Sex, F/M</td>
<td>10/7</td>
<td>3/15</td>
<td>0.0258</td>
</tr>
<tr>
<td>EASI score, mean ± SD</td>
<td>N/A</td>
<td>Pre-Rx 5.6 ± 5.8, Post-Rx 2.0 ± 8.2</td>
<td>1.58E–07</td>
</tr>
</tbody>
</table>

EASI, Eczema Assessment Severity Index; F, female; M, male; N/A, not applicable; Rx, treatment; SD, standard deviation.
genome, using STAR (open source aligner).\textsuperscript{52} Mapped sequencing reads were assigned to genomic features using the featureCounts function. Counts were transformed to log scale by voom transform.\textsuperscript{53}

For all samples (AD and healthy), fold changes (FCHs) were estimated, and hypothesis testing was conducted using contrasts under the general framework for linear models in the limma package. P values were adjusted for multiple hypotheses using the Benjamini-Hochberg procedure, controlling for false discovery rate (FDR). Proteins with an [FCH] of greater than 1.5 and an FDR of less than 0.05 were considered differentially expressed. In addition, a nonparametric multivariate previously published µ-stat approach\textsuperscript{55} was used to integrate multiple skin biomarkers. This approach uses U-statistics for scoring multivariate ordinal data and then correlates them with the outcome in question.\textsuperscript{42}

Gene set overexpression analysis was performed with XGR software using canonical/KEGG/Reactome/BioCarta pathways,\textsuperscript{55–58} with an FDR of less than 0.05.

RESULTS

RNA-seq was used to evaluate expression of immune and barrier genes in lesional and nonlesional tape-stripped skin from 18 moderate to severe AD patients, before and after dupilumab therapy, and from 17 healthy adults. We were able to extract RNA and perform RNA-seq from all samples. The mean percent change in EASI with dupilumab was 72.9% ($P < 0.001$, from 20.7 to 5.6; Table 1). Using criteria of an [FCH] of greater than 1.5 and an FDR of less than 0.05, we identified at baseline 6745 genes (up: 3994, down: 2751) that were differentially expressed in lesional AD versus controls and significantly decreased across AD tissues at baseline with treatment. They largely did not attain significant changes with treatment.

Because we observed changes in many genes that are interrelated, we also performed a gene set variation analysis using previously published AD-related pathways,\textsuperscript{21,50,59–61,64} including T-cell and Th1/2 pathways, which were both significantly enriched in AD tissues versus controls and significantly decreased with treatment ($P < 0.05$; Fig. 3).

Tape-Strips From AD Patients Treated With Dupilumab Showed Improvement in the Immune AD Signature

We further evaluated how dupilumab treatment modulated expression in lesional and nonlesional tape-stripped skin from 18 moderate to severe AD patients, before and after dupilumab therapy, and from 17 healthy adults. We were able to extract RNA and perform RNA-seq from all samples. The mean percent change in EASI with dupilumab was 72.9% ($P < 0.001$, from 20.7 to 5.6; Table 1). Using criteria of an [FCH] of greater than 1.5 and an FDR of less than 0.05, we identified at baseline 6745 genes (up: 3994, down: 2751) that were differentially expressed in lesional AD versus controls and significantly decreased across AD tissues at baseline with treatment. They largely did not attain significant changes with treatment.

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Atopic Dermatitis Barrier Defects Show Improvement After Dupilumab Therapy

We observed that genes related to terminal differentiation (periplakin [PPL], PSORS1C2, scillein [SCEL]), keratins (KRT77, KRT79), and tight junctions (GJB3, GJB5) were significantly downregulated at baseline, with significant or trending for significant increases after dupilumab treatment as depicted in a heatmap (Fig. 2 and Supplemental Table 2, http://links.lww.com/DER/A65, http://links.lww.com/DER/A66). Filaggrin was significantly decreased across AD tissues at baseline and increased after treatment, achieving significance by $P$ values in lesional skin ($P < 0.05$). Many lipid metabolism markers, previously associated with the barrier defect in AD,\textsuperscript{16,43,48,65,66} were significantly decreased at baseline (FA2H, SPTLC3, DHCR7, PNPLA3) and increased with treatment. Although epidermal hyperplasia markers (SERPINB3, KRT16, MK67, S100As) were upregulated at baseline, they largely did not attain significant changes with treatment. Peroxisome Proliferator Activated Receptor Gamma (PPARG), a transcription factor involved in lipid metabolism and systemic inflammation,\textsuperscript{67,68} was significantly increased at baseline and showed significant decreases after treatment in nonlesional AD.

A gene set variation analysis of a previously published barrier gene subset\textsuperscript{21,64,69} showed significant downregulation in both lesional and nonlesional AD at baseline, with significant upregulation after treatment ($P < 0.05$; Fig. 3).

Changes in Immune and Barrier Biomarkers Correlate With Disease Improvement With Treatment

Significant positive univariate correlations with expressions of key T(H)17/22-related genes (IL-12B/IL12/23p40, CCL20; \(R > 0.5, P < 0.05\)) were observed in lesional skin (Table 2). Epiregulin, a marker associated with epidermal hyperplasia,\(^7\) also positively correlated with EASI improvement (\(R = 0.64, P = 0.005\)). The negative regulator IL-34 inversely correlated with changes in EASI (\(R = -0.46, P < 0.05\)). Lipid (FABP7, AWAT1), tight junction (CDH20, CLDN10), and keratin (KRT79) genes (\(R < -0.4, P < 0.05\)) also negatively correlated with EASI changes. We also performed a multivariate correlation analysis using a \(\mu\)-stat approach,\(^4,5\) to integrate changes in lesonal and nonlesional biomarkers with changes in disease severity. This analysis resulted in much higher correlations than those seen in the univariate approach. Representative multivariate combinations (eg, epiregulin/PPARG/IL34), with

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**Figure 1.** Heatmap of immune genes. Heatmap of the 75 top differentially expressed immune genes in tape-stripped AD lesional and nonlesional skin at baseline and in response to dupilumab therapy. Criteria for differential gene expression include an |FCH| of greater than 1.5 and an FDR of less than 0.05. Table shows FCHs in nonlesional AD versus normal at baseline, lesional AD versus normal at baseline, lesional versus nonlesional AD at baseline, posttreatment versus pretreatment in nonlesional skin, posttreatment versus pretreatment in lesional skin, posttreatment lesional versus nonlesional skin, and posttreatment nonlesional skin versus normal. LS, lesional; N, normal; NL, nonlesional; Pre, pretreatment; Post, posttreatment. ***FDR < 0.001, **FDR < 0.01, *FDR < 0.05, +FDR < 0.1.
correlations approaching 0.8 (P < 0.001), are shown in Table 2, with additional combinations listed in Supplemental Table 7, http://links.lww.com/DER/A69.

Key Treatment-Response Biomarkers Are Detected Using Tape-Strips

We next compared our current tape-strip data with a previously published gene expression (using microarrays and real-time polymerase chain reaction) biopsy study that was a phase II clinical trial for patients treated with dupilumab (Supplemental Tables 8–9, http://links.lww.com/DER/A70, http://links.lww.com/DER/A71).21 Analysis of tape strip specimens detected similar changes in many immune and barrier AD biomarkers (eg, CCL17, CCL18, CCL22, CD3G, KRT77) to those seen in skin biopsy specimens (Supplemental Tables 8–9, http://links.lww.com/DER/A70, http://links.lww.com/DER/A71). Tape-strips even detected larger changes compared with biopsies in some inflammatory (JAK2, STAT4, STAT6, Figure 2. Heatmap of epidermal barrier genes. Heatmap of differentially expressed epidermal barrier–related genes, using criteria of an |FCH| of greater than 1.5 and an FDR of less than 0.05. Table shows FCHs in nonlesional AD versus normal at baseline, lesional AD versus normal at baseline, lesional versus nonlesional AD at baseline, posttreatment versus pretreatment in nonlesional skin, posttreatment versus pretreatment in lesional skin, posttreatment lesional versus nonlesional skin, and posttreatment nonlesional skin versus normal. LS, lesional; N, normal; NL, nonlesional; Pre, pretreatment; Post, posttreatment. ***FDR < 0.001, **FDR < 0.01, *FDR < 0.05, +FDR < 0.1.
CD4, CD1A, FCER1A) and barrier-related biomarkers (PPL, PSORS1C2, FA2H; Supplemental Tables 8–9, http://links.lww.com/DER/A70, http://links.lww.com/DER/A71).

On the other hand, analysis of skin biopsy specimens more robustly detected changes with treatment in some TH17/TH22 (IL-12B/IL12/23p40, IL-23A/IL-23p19, PI3, CXCL1, CXCL2) and epidermal hyperplasia genes (KRT16, SERPINB3, MKi67, S100As; Supplemental Tables 8–9, http://links.lww.com/DER/A70, http://links.lww.com/DER/A71).

**Tape-Strips Detect Modulation of Additional Pathways With Treatment**

For a broader perspective on the effect of dupilumab treatment in AD patients captured by tape-strips, all of the differentially expressed genes that were detected in lesional AD skin after versus before treatment were analyzed using function-based pathway databases (canonical/KEGG/Reactome/BioCarta), revealing significant modulation of both expected and novel pathways (FDR < 0.05). The top downregulated pathways included those related to cytokine and chemokine signaling, T-cell activation (eg, CD40L signaling), adaptive/innate immunity (eg, IL-8–mediated events), and hallmark ILs of AD (eg, IL-4, IL-22, and IL-12 pathways; Supplemental Fig. 1A, http://links.lww.com/DER/A72). We also detected downregulation of DC activity in TH1 and TH2 development and mast cell activation (eg, Fcɛ receptor I signaling). Novel findings included downregulation of several complement pathways (eg, classic complement pathway and initial triggering of complement). Moreover, this analysis revealed that dupilumab treatment in atopic patients leads to downregulation of several vascular-related pathways, such as cell surface interactions at the vascular wall, platelet activation and aggregation, and platelet-derived growth factor and thromboxane A2 receptor signaling. On the other hand, we detected enrichment of epidermal barrier–related pathways, such as those involved in stabilization and expansion of the E-cadherin adherens junction, gap junction trafficking, lipid and lipoprotein metabolism, fatty acid/triacylglycerol/ketone body metabolism, and cholesterol biosynthesis (Supplemental Fig. 1B, http://links.lww.com/DER/A72).

**DISCUSSION**

Skin biopsy studies have helped elucidate the immune and barrier pathomechanisms underlying AD and have been instrumental in defining biomarkers of treatment response. However, thus far, analysis of skin biopsy specimens have been used primarily in early...
phase clinical trials, and their invasive nature has limited their use in larger-scale studies as well as in real-life settings. There is an unmet need for minimally invasive approaches to provide meaningful treatment-response biomarkers in skin. Tape-strips are emerging as an alternative to full-thickness skin biopsy specimens to profile lesional and nonlesional skin in adults and children with AD and beyond. However, although our group recently used Olink proteomics to analyze changes in approximately 350 proteins with dupilumab treatment, global transcriptomic analysis in response to systemic immune antagonists, including those targeting specific cytokines, has not yet been explored using tape-strips from AD patients.

Here, we present the first comprehensive molecular profiling study using RNA-seq that characterizes tape-strips obtained from lesional and nonlesional skin of moderate to severe AD patients before and after 16 weeks of dupilumab treatment in a real-life setting. Our data show that tape-strips accurately captured key immune and barrier response biomarkers to dupilumab treatment in AD (eg, CCL17, CCL18, PSORS1C2, SCEL) similar to skin biopsies. Some markers (eg, IAK2, STAT4, CD4, FCER1A, PPL, FA2H) showed even larger differences to those seen in skin biopsy specimens, but a few epidermal hyperplasia–related genes (MKI67, KRT16, S100As) demonstrated larger modulation in biopsies. As in clinical trial studies with dupilumab, Tg11-related genes were not significantly changed in tape-strips. Furthermore, analysis of tape-strips more robustly detected biomarker changes at week 16 in nonlesional AD skin compared with those observed in skin biopsies. Analysis of Tape-strips have been previously suggested to better capture some epidermal differentiation biomarkers (ie, PPL) in nonlesional AD skin, possibly because of the focused production of these markers in outer skin layers, whereas those biomarkers may be diluted in whole skin biopsies. Our data, derived from tape-strips is also the first to show that nonlesional skin becomes more similar to skin from healthy individuals after dupilumab treatment with nonsignificant differences in key terminal differentiation, tight junction, and lipid products (eg, filaggrin, CDH20, FA2H), highlighting tape-strips as a robust way of looking at nonlesional skin and thus potentially rendering this skin tissue more informative for the study of AD.

The analysis of the effect of dupilumab was very evident in tape-strips for CD1A and FCER1A, markers of inflammatory dendritic epidermal cells, whereas changes in these markers did not reach significance in skin biopsy specimens. Inflammatory dendritic epidermal cells are highly expressed in lesional epidermis but are at low levels or absent in nonlesional and normal epidermis. Analysis of tape-strips may be more suitable at detecting these markers after dupilumab therapy compared with skin biopsy specimens because as the disease resolves, there are larger decreases of these markers in the epidermis compared with the dermis. In whole tissue specimens, this change is diluted by the large dermal component, whereas tape-strips only sample up to the granular layer, allowing for more selective detection of these markers in the epidermal compartment.

Although many genes significantly modulated in lesional and nonlesional skin were shared (eg, MMP12, CD1B, FCER1A, CXCL16, PPL, SCEL), there were differences in expression of some markers between the 2 skin types after treatment. For example, markers of T cells and T-cell migration/activation (C3D3, C3D5, CDS, CCR7, ICOS, CD86) were most prominently suppressed by dupilumab in nonlesional skin, although they did not reach significance in lesional skin. This is notable, as this was the only immune set of genes that was more decreased in nonlesional compared with lesional skin. Indeed, tape-strips have been previously suggested to more robustly capture the disease activity in the superficial portions of nonlesional AD skin, possibly because of the thinner epidermis of nonlesional skin. Likewise, several barrier-related genes, such as annexins (ANXA5/6), were significantly downregulated only in nonlesional skin, whereas PSORS1C2 and some lipid metabolism genes (eg, FA2H, DHCR7, PNPLA3) were only significantly upregulated in lesional skin.

We also identified correlations between changes in biomarkers and clinical severity (EASI), with many of these markers found to be correlated with treatment response in tape-strips for the first time. Notably, decreases in T117/T122 genes (eg, IL-12B/IL-12/23p40, CCL20) significantly correlated with improvement in disease severity, similar to the biopsy study with dupilumab. Negative regulators (IL-34 and IL-1F10) were decreased at baseline and showed strong upregulation with dupilumab. Interleukin 34, a mediator that helps regulate genes involved in lipid metabolism and systemic inflammation, was significantly decreased in nonlesional skin with dupilumab, and the change was also correlated with EASI improvement, representing a potentially novel treatment response biomarker. When integrating many of the differentially expressed genes that were detected with the analysis of tape-strips in AD lesional skin after versus before dupilumab therapy by using comprehensive signaling pathway databases, we found significant decreases in various new pathways, especially those related to vascular processes, platelets, and complement activation, in addition to significant changes in expected immune (eg, IL-4) and barrier (eg, lipid, gap junction) pathways. These data add to the growing understanding of the systemic nature of AD and are in line with studies that suggested the ability of dupilumab to decrease many inflammatory and vascular-related markers. Thus, analysis of tape-strips are able to elucidate new pathways that may potentially expand the understanding of AD pathogenesis and therapy.

This study had few limitations. The sample size was relatively small, and although we detected significant comparisons, a larger cohort would be desirable. In addition, because this was a real-life study, we were unable to concomitantly obtain skin biopsy specimens in the
same cohort and to evaluate dupilumab-induced transcriptomic changes in tape-strips from a placebo group. Because tape-strips are able to only remove keratinocytes up to the granular layer, it is possible that some epidermal hyperplasia genes may not be optimally detected by tape-strips because of their more basal/suprabasal localization. Nevertheless, many inflammatory and epidermal markers were similarly or even more highly captured in tape-strips compared with skin biopsies. Furthermore, here, we used RNA-seq data, whereas the dupilumab biopsy study was performed on microarrays. Nonetheless, this study detected larger FCHs in many markers, possibly because of reduced dilution of the epidermal markers in tape-strips compared with full-thickness biopsies.

Overall, analysis of tape-strips are able to track molecular responses in skin during treatment with dupilumab in AD patients and to provide treatment-response biomarkers that correlate with clinical improvement. Tape-strips may enable serial skin sampling in AD and beyond, facilitating development of a personalized medicine approach for inflammatory skin conditions.

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