Intra-patient comparison of atopic dermatitis skin transcriptome shows differences between tape-strips and biopsies

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Abbreviations

AD: Atopic dermatitis

CCL: CC chemokine ligand

CXCL: C-X-C Motif Chemokine Ligand

DC: Dendritic cell

DEG: Differentially expressed gene

FDR: False discovery rate

IDEC: Inflammatory dendritic epidermal cell

IGA: Investigator's Global Assessment

IL: Interleukin

LC: Langerhans cell

Th: T-helper

TSS: Total Skin Score

ABSTRACT

Background

Our knowledge of etiopathogenesis of atopic dermatitis (AD) is largely derived from skin biopsies, which are associated with pain, scarring and infection. In contrast, tape-stripping is a minimally invasive, nonscarring technique to collect skin samples.

Methods

To construct a global AD skin transcriptomic profile comparing tape-strips to whole skin biopsies, we performed RNA-seq on tape-strips and biopsies taken from the lesional skin of 20 moderate-to-severe AD patients and the skin of 20 controls. Differentially expressed genes (DEGs) were defined by fold-change (FCH)[?]2.0 and false discovery rate$<$0.05.

Results

We detected 4,104 (2,513 Up; 1,591 Down) and 1,273 (546 Up; 727 Down) DEGs in AD versus controls, in tape-strips and biopsies, respectively. Although both techniques captured dysregulation of key immune genes, tape-strips showed higher FCHs for innate immunity (IL-1B, IL-8), dendritic cell (ITGAX/CD11C, FCER1A), Th2 (IL-13, CCL17, TNFRSF4/OX40), and Th17 (CCL20, CXCL1) products, while biopsies showed higher up-regulation of Th22 associated genes (IL-22, S100As) and dermal cytokines (IFN-γ, CCL26). Itch-related genes (IL-31, TRPV3) were preferentially captured by tape-strips. Epidermal barrier abnormalities were detected in both techniques, with terminal differentiation defects (FLG2, PSORS1C2) better represented by tape-strips and epidermal hyperplasia changes (KRT16, MKI67) better detected by biopsies.

Conclusions

Tape-strips and biopsies capture overlapping but distinct features of the AD molecular signature, suggesting their respective utility for monitoring specific AD-related immune, itch, and barrier abnormalities in clinical trials and longitudinal studies.

INTRODUCTION

Atopic dermatitis (AD) is a common inflammatory skin disease affecting up to 10% of adults and 20% of children worldwide.[1,2] Our knowledge of AD pathomechanisms is largely derived from molecular studies in skin biopsies that demonstrate primarily Th2/Th22 inflammation with variable Th1/Th17 skewing, as well as epidermal barrier dysregulation, including epidermal hyperplasia and abnormalities in terminal differentiation, tight junctions, and lipid biosynthesis/metabolism.[3-12] While skin biopsies were instrumental to deciphering disease characteristics, they are associated with pain, scarring, and infection risk, restricting their use in large-scale clinical trials and longitudinal studies, which are critical for adults and even more in children.[13-15] Tape-strips are a minimally invasive, nonscarring approach to collect skin samples to the level of the upper stratum granulosum.[16] Recent studies have demonstrated significant utility of tape-strips in profiling AD skin in both adults and children,[6,16-30]and in monitoring disease activity in response to treatment.[31-34]

Comprehensive molecular intra-patient comparison of tape-strips to full-thickness biopsies is currently lacking. Moreover, there is no detailed comparison of immune, barrier, and itch-related markers between the two techniques. [16,35,36] Several studies described differences between tape-strips and biopsies using a limited panel of markers. Andersson et al. analyzed only 17 immune markers in AD skin and noted that CCL17 and markers of innate immunity were significantly up-regulated and correlated with disease activity in tape-strips but not in same-cohort biopsies.[36] Simonsen et al. assessed 10 cytokines in AD lesions and noted greater up-regulation in the innate immunity marker IL-1B in tape-strips, while dysregulation of Th2 markers and IFN-γ were only detected in biopsies.[35] Of note, this study measured mRNA expression in biopsies while proteomics was performed in tape-strips. Kim et al. measured mRNA of 5 epidermal differentiation markers in tape-strips, reporting positive correlations with protein levels in biopsies.[16] Utilizing RNAseq, Sølberg et al. reported that structural genes like keratins were higher in biopsies while immune markers IL-8 and IL-36A were higher in tape-strips.[18] Dyjack et al. analyzed tape-strips from non-lesional AD skin, noting better representation of cornification genes in tape-strips compared to biopsies.[37] These studies enzymatically separated the epidermis and dermis in biopsies, potentially overlooking important pathogenic features within the entire AD skin transcriptome.

Herein, we present a global RNA-seq intra-patient profiling of tape-strips and whole-skin biopsies in moderate-to-severe AD as compared to healthy subjects. Our data highlight features that are better captured by tape-strips, including Th2 polarization, itch pathway activation, and terminal differentiation impairment, whereas Th22 changes and epidermal hyperplasia are more pronounced in biopsies. These findings suggest that tape-strips and biopsies each preferentially detect unique disease characteristics, which should be considered when selecting skin sampling approaches in clinical trials or longitudinal studies.

METHODS

Study population and characteristics

We enrolled 20 adults with moderate-to-severe AD (15 females and 5 males, mean age 31 years) and 20 healthy volunteers (11 females and 9 males, mean age 40.3 years) under institutional review board-approved protocols (**Table 1**). Clinical severity scores were measured using a 5-grade AD Investigator's Global Assessment (IGA) score (mean score 2.6, **Table 1**). Exclusion criteria included immunodeficiency, use of biologics within the previous 12 weeks, use of systemic steroids, use of immunosuppressants or phototherapy within the previous 4 weeks, and use of any topical medication within the previous 2 weeks.

Tape strip collection, biopsy collection, RNA extraction, and RNA-seq

Twenty consecutive large D-Squame tape-strips (CuDerm, Dallas, Texas) were taken from the lesional skin of AD patients and from the skin of healthy controls. The first tape-strip was discarded, and remaining samples were frozen at –80°C as previously described.[17,38] Four millimeters skin biopsies were taken from the lesional skin of AD patients and from the skin of controls (same subjects as tape-strips). RNA was extracted using an miRNAeasy Mini Kit (Qiagen, Hilden, Germany) as previously described.[39] In tape-strips, RNA AmpliSeq libraries were constructed with the Ion AmpliSeq Transcriptome Human Gene Expression Kit by using 5 ng of RNA per sample. RNA-seq libraries were pooled and sequenced on the Ion S5 XL system sequencer with Ion 550 Chips (Thermo Fisher, Waltham, Mass). In biopsies, libraries were generated using TruSeq Stranded mRNA Library Prep kit (Illumina). Next generation sequencing was performed on Illumina NovaSeq6000 (Illumina Inc., 100 cycles, single-read sequencing).

Statistical analyses

Statistical analyses were performed by using the statistical language R (www.R-project.org). The quality of samples was assessed using FastQC. Each sample was aligned to the human reference genome by using STAR (open-source aligner).[40] Mapped sequencing reads were assigned to genomic features using the featureCounts function. Counts were transformed to log scale by voom-transform and fit in a linear model.[41] Fold-changes (FCHs) were estimated, and hypothesis testing was conducted by using contrasts under the general framework for linear models in the limma package. P-values were adjusted for multiple hypotheses by using the Benjamini-Hochberg procedure, controlling for false discovery rate (FDR). Genes with FCH[?]2 and FDR<0.05 were considered DEGs.

RESULTS

Using RNA-seq, we provided a global transcriptomic profile of tape-strips and biopsies taken from the lesional skin of moderate-to-severe AD patients, and from healthy control skin (n=20 each) (**Figure 1A**). Sample recovery rates were 19/20 (95%) in AD and 19/20 (95%) in controls for tape-strips and 100% of AD and controls for biopsies.

Tape-strips and biopsies capture key AD-related immune alterations

Using FCH[?]2 and FDR<0.05 criteria, tape-strips and biopsies, respectively, detected 4,104 (2,513 Up; 1,591 Down) and 1,273 (546 Up; 727 Down) DEGs in AD versus controls (**Figure 1B**). The common phenotype between tape-strip and biopsy groups comprised of 536 DEGs (310 Up; 226 Down).[42] The tape-strip group had 3,568 unique DEGs (2,203 Up; 1,365 Down), whereas biopsies had 737 unique DEGs (230 Up; 292 Down) as depicted in the Venn diagram in **Figure 1B** .

Using a previously published immune-data set,[17,29,42-46] we identified inflammatory genes that were differentially expressed in AD versus controls in at least one comparison (**Figure 2, Table E1**). The immune-related DEGs with the highest FCHs are shown in a heatmap in **Figure 2A-B** and a visual word cloud of representative immune markers, where the size of the letters represents the magnitude of the FCHs in AD versus healthy skin, is depicted in **Figure 2C-D** . Both tape-strips and biopsies showed significant up-regulation in key products involved in general inflammation (MMP12), innate immunity (IL-6), T-cells/T-cell activation (CD2, CD3D/E/G, CD4, CD5, CD28, ICOS, GZMA), dendritic cell (DC) activation and migration (FCER1G, ITGAM/CD11B, ITGAX/CD11C, CD80, CXCR4), regulatory markers (IL-10, CTLA4, FOXP3, CD274), Th2 (IL-13, CCL13, CCL17, CCL18, CCL22, IL-4R, TNFRSF4/OX40, OSM, CCR4), Th1 (IL-12B, CXCL9, CXCL10, MX1, OASL), Th17 (CCL20, CXCL1, IL-19, IL-20, IL-36A, PI3), and antimicrobial peptides (CAMP/LL37) (**Figure 2, Table E1**). Both techniques also demonstrated significant down-regulation of negative regulators IL-34, IL-37, IL-1F10. These markers all notably showed greater FCHs in tape-strips than in biopsies, particularly for select markers of T-cells/T-cell activation (CD3D, CD3E, GZMA), negative regulation (CD274, IL-34), DC activation and migration (FCER1G, CD80, ITGAM/CD11B, ITGAX/CD11C, CXCR4), and Th2 (IL-13, CCL17, TNFRSF4/OX40, CCR4, OSM), where the FCHs between AD and controls were at least 5-fold in tape-strips compared to biopsies (**Figure 2A and C**). Moreover, multiple additional genes involved in innate immunity or general immune activation (IL-1B, IL-8, PDE4A, PDE4B, PTPRC, NLRP3, TLR4), tissue resident memory T-cells (CD69), DCs (CD83, CD86, CD1A,

CD1C, CD1E, FCER1A, CD207/langerin, IL-3RA, IRF4),[9,47,48] macrophages (RNASE1, MS4A4A, F13A1, CECR1), Th1/Th2 (CCL3), and Th17 (CXCL2, CXCL3, GPR183, TGM2, DUSP4) were uniquely up-regulated in tape-strips, but not biopsies (**Figure 2, E1,Table E1**). Conversely, the Th1-defining cytokine IFN-γ, CCL26, a Th2 chemokine, and CCL19, a chemokine involved in T-cell trafficking, were uniquely significantly up-regulated in biopsies. Th22 and Th17/Th22-related genes (IL-22, IL-26, S100A7, S100A8, S100A9, DEFB4B, and LCN2) showed higher or unique up-regulation in biopsies compared to tape-strips. Collagens (COL6A5, COL6A6, TNC) implicated in driving AD inflammation were also uniquely up-regulated in biopsies (**Figure E1, Table E1**).[9]

Tape-strips preferentially captured up-regulation of itch-related gene products

Gene products associated with pruritus and nociception[49-55] that were dysregulated in either tape-strips or biopsies are shown in **Figure 3** . IL-31 and OSM drive pruritus via interactions with the IL-31RA and OSMR heterodimer on sensory neural cells.[56-61] IL-31, OSM, and OSMR were either uniquely upregulated or showed higher FCHs in AD versus controls in tape-strips compared to biopsies. STAT3, which regulates IL-31 signaling showed a similar trend.[56]

Transient receptor potential (TRP) channels (TRPV2, TRPV3, TRPM2)[55] demonstrated greater up-regulation in tape-strips compared to biopsies, while TRPV6 showed greater down-regulation in tape-strips **(Figure 3)** . TRPV3 activation was shown to induce a pruritogenic pathway involving SERPINE1, PLAUR/u-PAR, and TLR2,[62,63] which were either uniquely (PLAUR/u-PAR, TLR2) or markedly more (SERPINE1) increased in tape-strips than in biopsies (**Figure 3, E1, Table E1)** . Other genes with higher up-regulation in tape-strips than biopsies include cathepsins (CTSS, CTSL, CTSB), serine protease inhibitor SERPINB1, CGRP receptor component RAMP1,[54] and phospholipase PLCB3, a neuronal marker required for histamine and serotonin-mediated pruritus (**Figure 3, Table E1**).[51-53]Histamine-dependent itch markers (AOC1, HDC) and PTGER2, which is involved in vasodilation and cytokine secretion by endothelial cells,[49,50] were only significantly up-regulated in tape-strips, while the histamine receptor HRH3 was up-regulated only in biopsies.[64] The kallikreins KLK6 and KLK9, which are enriched or restricted to the stratum granulosum, were comparably up-regulated in tape-strips and biopsies, while KLK7, which is highest in the basal layer, was only up-regulated in biopsies.[65]

Epidermal barrier alterations in tape-strips compared to biopsies

We next evaluated DEGs between AD and controls in either tape-strips or biopsies among a previously published epidermal barrier gene-subse[4,11,30,66] (**Figure 4)** . While terminal differentiation (FLG2, SCEL, PSORS1C2, LCE5A) and keratin (KRT77, KRT79) products were significantly decreased in both techniques, greater decreases were observed in tape-strips. AKR1C3, which is expressed by differentiated keratinocytes, was the most down-regulated gene unique to tape-strips (**Figure E1**). Significant decreases in gap junction genes (GJB3, GJB5) were only seen in tape-strips (**Figure 4, Table E1**). Tight junction components like claudins and cadherins (CLDN8, CDH12)[67] were comparably suppressed in tape-strips and biopsies, while CLDN1 was uniquely significantly decreased in biopsies. Markers of epidermal hyperplasia/proliferation (KRT6A, KRT6B, KRT16, KRT17, MKI67, AKR1B10, AKR1B15) and cornification (SPRR1A, SPRR2C, SPRR3) showed higher or unique increases in biopsies.

There was considerable variability in lipid biosynthesis and metabolism genes. PNPLA3, GAL, FA2H, FABP7, DGAT2, ACER1, ORMDL3, and DHCR7 were significantly decreased in both, with slightly greater FCHs in tape-strips, and DEGS2 and SPTLC3 were significantly down-regulated only in tape-strips. However, several other lipid-related genes (ELOVL3, FAR2, FADS2, ACOX2) were uniquely down-regulated in biopsies. A few genes (AGPAT3, FADS1, ELOVL5, SOAT1, LPIN1, PPARG) were decreased in biopsies, but increased in lesional tape-strips.

Enrichment of key immune, pruritus and barrier-related pathways

We used gene set variation analysis (GSVA) on the previously published AD transcriptome (MADAD), and Th1-, Th2- and Th17/Th22- as well as pruritus and barrier-related genes, to evaluate how the two

approaches compare.[29,42,68] We observed similar significant enrichment in all key analyses in both biopsies and tape-strips analyses (**Figure 5**).

DISCUSSION

We present the most comprehensive intra-patient transcriptomic comparison of tape-strips and biopsies in moderate-to-severe AD to date. We previously developed a framework for using tape-strips to measure mRNA and protein in AD in both pediatric and adult populations, including for monitoring skin activity with topical or systemic treatment.[29,30,69,70] Here, we provided insights into the common AD phenotype detected by both tape-strips and biopsies as well as potential differences between these approaches in detecting specific disease features. Past reports comparing tape-strips and biopsies in AD are limited either by a small panel of markers,[16,35,36] separation of epidermal and dermal layers in biopsies,[18,37,71] or focus on non-lesional skin.[37,71] An intra-patient, RNA-seq profiling of active AD lesions comparing tape-strips to full-thickness biopsies, thus capturing the full spectrum of immune, barrier, and pruritus-related molecular abnormalities is not available.

Our study showed that both tape-strips and biopsies captured key molecular immune abnormalities of AD, including T-cell and DC activation, innate immunity, Th2/Th1/Th17/Th22, and attenuated negative regulation to varying degrees. However, tape-strips and biopsies also showed some distinct biomarker profiles, with some notable biomarkers differentially expressed in only one of the two techniques. Overall tape-strips outperformed biopsies for most T-helper axes, with greater differentiation from controls for Th2 (IL-4R, IL-13, IL-31, CCL17, TNFRSF4/OX40), Th17 (CCL20, CXCL1, CXCL2), innate immunity (IL-1B, IL-8, TNF), some Th1-related chemokines (CXCL10, CCL3), and negative regulator markers (IL-34, IL-37). IL-34, in particular, was shown to distinguish AD from healthy skin with almost perfect accuracy in tape-strips,[30] a property that is likely unique to tape-strips given the >35x higher fold-change between AD and controls in tape-strips compared to biopsies. These findings suggest that chemokines that promote Th2/Th17 differentiation and their defining cytokines are found in high concentration in the upper epidermis, whereas their expression may be diluted in larger biopsy samples. Tape-strips also better captured DC-related products, including myeloid DCs (ITGAM/CD11B, ITGAX/CD11C), LCs, and inflammatory DCs (CD1A, FCER1A, CD207/langerin), mainly localized to the epidermis,[48,72,73] which are major sources of chemokines. Inflammatory DCs correlate with clinical AD severity and drive Th2 skewing,[74,75] representing a potential pathogenic cell type in AD with better detection by tape-strips.

In contrast, biopsies demonstrated higher fold-changes than tape-strips for hyperplasia related cytokines and chemokines, including the Th22 cytokine IL-22 and the S100As that are Th17 and Th22 co-regulated,[76] suggesting that biopsies may be more ideal for evaluating hyperplasia markers. IL-22 is primarily expressed in the dermis during inflammatory states,[77,78] while S100As are produced by suprabasal keratinocytes that are likely below the depth of tape-stripping.[9] Both promote keratinocyte proliferation and epidermal hyperplasia.[77] IFN-γ, the main Th1 cytokine, and CCL26, perhaps the best biomarker of therapeutic response to dupilumab, are additional AD biomarkers primarily localized to the dermis[78,79] and accordingly showed dysregulation only in biopsies. Markers of an inflammatory dermal fibroblast population that may orchestrate lymphocyte migration to secondary lymphoid organs and Th2 immunity, including COL6A5, COL6A6, TNC, and CCL19,[9] were also predictably solely up-regulated in biopsies. Of note, many markers that are more prominently detected by tape-strips (IL-4R, IL-13, IL-31, TNFRSF4/OX40) or biopsies (IL-22) are therapeutic targets for AD that are either FDA-approved or under investigation,[80-88] reinforcing that although both techniques can detect molecular changes in AD in response to therapy,[80,82,85,86] the type of therapeutic target may dictate the appropriate sampling technique.

Pruritus, the cardinal clinical feature of AD significantly impacting patients' quality of life,[89-91] is orchestrated by complex cross-talk between immune cells, neurons, and keratinocytes.[51,56,63,65,88,92-105] One of the advantages of tape-strips over biopsies was the ability to better detect changes in the neuroimmune pathways mediating pruritus. For example, the Th2 cytokine IL-31, perhaps the key driver of pruritus in AD,[56,88,92-95] was uniquely up-regulated in tape-strips, with similar trends in OSM and OSMR, which are part of the same itch pathway and directly interact with IL-31.[56]STAT3, which mediates IL-31-induced neuronal overgrowth

contributing to skin sensitivity to minimal stimuli,[93] was also dysregulated only in tape-strips. Several transient receptor channel components (TRPV2, TRPV3, TRPM3) showed more pronounced dysregulation in tape-strips, likely owing to their expression by sensory neurons, as well as keratinocytes and/or immune cells.[106,107]Among these, TRPV3 is most abundant on keratinocytes,[106] and has been shown to be increased in lesional AD,[62] and promote a pro-inflammatory cascade via the NF-κB pathway.[96] In contrast, TRPV6, which is the only down-regulated TRP gene, with more negative fold-changes in tape-strips than biopsies, is a pre-requisite for keratinocyte entry into differentiation.[100] As an example of the complex intercellular interactions that drive itch, IL-31-induced up-regulation of TRPV3 was proposed to induce keratinocyte release of SERPINE1, which then directly activates PLAUR/u-PAR in sensory neurons to perpetuate a cycle of itch and neuroinflammation via the innate immune system.[63,108] Accordingly, SERPINE1 and PLAUR/u-PAR were also exclusively dysregulated in tape-strips. Overall, given the large contribution of pruritus to the burden of AD,[89-91] the ability to measure the itch signature of AD before and after treatment through minimally invasive approaches, such as tape-strips, which in fact outperformed biopsies, is critical.

In addition to immune/neuroimmune dysregulation, tape-strips and biopsies also preferentially captured distinct aspects of epidermal barrier impairment. Although markers of terminal differentiation[109-111] (FLG2, SCEL, PSORS1C2) were decreased in both techniques, at least two-fold greater changes between healthy and lesional AD were seen in tape-strips. In contrast, biopsies showed more pronounced up-regulation of markers of epidermal hyperplasia (KRT6A, KRT16, MKI67), which are primarily expressed in the basal and immediately suprabasal layers.[112], Tight junction components, which are variably distributed throughout the epidermis,[78] were largely attenuated in AD in both techniques, with some differences. For example, down-regulation of CLDN1, which is expressed at all suprabasal layers and has been described as an AD susceptibility gene with inverse correlations to Th2,[113] was only detected in biopsies. In contrast, the gap junction components (GJB3, GJB5), which are primarily expressed in the granular layer,[114] were only significantly decreased in tape-strips. Lipid biosynthesis/metabolism abnormalities, which are partly driven by Th2 in AD,[115] were observed in both tape-strips and biopsies in line with prior reports,[7,86,115-119] but also showed differences between the two techniques. For example, genes involved in the biosynthesis of ceramides, the main lipid constituent of the stratum corneum (DGS2, SPTLC3),[120] were only decreased in tape-strips, while products related to phospholipid synthesis (AGPAT3, LPIN1), which are more common in the lower epidermis,[120] were only down-regulated in biopsies. The inclusion of lipids from the sebocytes of sebaceous glands in biopsies but not tape strips, which also contribute to barrier function,[121] may further contribute to differences in lipid-related genes between the two techniques. FAR2, which regulates synthesis of wax esters exclusively produced in sebaceous glands,[122] and FADS2, which regulates production of sapienate, the most abundant fatty acid in sebum,[123]were down-regulated only in biopsies. Overall, tape-strips and biopsies each preferentially capture gene alterations that complement each other to constitute the defective barrier phenotype characteristic of AD.

Our study has some limitations. Non-lesional biopsies were not available for this cohort, preventing a parallel intra-patient comparison of non-lesional tissues analyzed by both approaches. Nevertheless, prior studies demonstrated that tape-strips capture the non-lesional AD phenotype.[29,37,69,71,124] Additionally, our analyses were performed on a primarily adult Caucasian population. Future analysis should also include other age groups and ethnic backgrounds.

In summary, this is the first global, intra-patient, full-thickness molecular profiling of lesional skin from moderate-to-severe AD that compares sampling by tape-strip versus biopsies. Although both capture the main immune and barrier abnormalities of AD, tape-strips may be the preferred technique for Th2/Th17, innate immunity, DC, pruritus, and terminal differentiation related markers. Biopsies may be more appropriate for evaluating genes below the granular layer, including markers of epidermal hyperplasia, dermal cytokines (IFN-γ, IL-22), and inflammatory dermal populations like fibroblasts. The choice of appropriate sampling technique should be determined considering which specific immune or barrier features are of greatest importance to the investigation. Still, this study highlights that tape-strips are not only adequate but can even outperform biopsies in detecting certain AD features, including several biomarkers that are therapeutic targets. Thus, tape-stripping provides a minimally invasive alternative or supplement to biopsies

in clinical trials and longitudinal studies with the potential to minimize biopsy-related complications and bolster patient participation in adult and pediatric AD studies.

Table 1. Demographics

	AD (n = 20)	**Controls (n =20)**
Age, mean ± SD	31.0 ± 13.8	40.3 ± 15.7
Sex, no. (%)		
Female	15	11
Male	5	9
Race, no. (%)		
White	18	20
African American	2	0
Asian	0	0
Clinical severity		
IGA, mean ± SD	2.6 ± 0.6	N/A

FIGURES LEGEND :

Figure 1. Study summary. (A) Study design. (B) Venn diagram of differentially expressed genes (DEGs) in tape-strip and biopsy groups by fold-change (|FCH|)>2 and false discovery rate (FDR)<0.05. Up-regulated (*red*) and down-regulated (*blue*) DEGs in AD versus control in each group depict shared versus unique genes across the two techniques.

Figure 2. Heatmap of immune-related genes. Heatmap of the top differentially expressed immune genes in tape-stripped (A), and biopsied (B) AD lesional skin. Fold-change (|FCH|)>2 and false discovery rate (FDR)<0.05. Tables show fold-changes in lesional AD versus normal skin. World Cloud diagram of immune DEGs of tape-strips (C) and biopsy (D) group. The size of the letters represents the magnitude of the FCHs in AD versus healthy skin. LS, Lesional. ***FDR<0.001, **FDR<0.01, *FDR<0.05, +FDR<0.1.

Figure 3. Heatmap of pruritus-relates genes. Heatmap of the differentially expressed pruritus-related genes in tape-stripped (A), and biopsied (B) AD lesional skin. Fold-change (|FCH|)>2 and false discovery rate (FDR)<0.05. Tables show fold-changes in lesional AD versus normal skin. LS, Lesional. ***FDR<0.001, **FDR<0.01, *FDR<0.05, +FDR<0.1.

Figure 4. Heatmap of barrier-related genes. Heatmap of the differentially expressed barrier-related genes in tape-stripped (A), and biopsied (B) AD lesional skin. Fold-change (|FCH|)>2 and false discovery rate (FDR)<0.05.Tables show fold-changes in lesional AD versus normal skin. LS, Lesional. ***FDR<0.001, **FDR<0.01, *FDR<0.05, +FDR<0.1.

Figure 5. Gene-set variation analysis (GSVA). Gene-set variation analyses (GSVA) of immune-related and barrier-related genes. Red bars represent means. *Black symbols* : significance of comparison to normal*; red symbols:* significance of comparison between lesional and non-lesional skin. MADAD, Meta-Analysis Derived AD transcriptome; LS, Lesional. ***P <0.001, **P <0.01, *P <0.05, +P <0.1.

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Figure 1

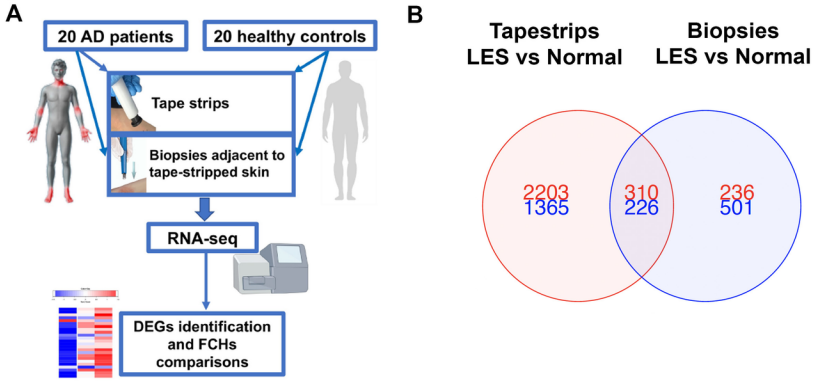

Figure 2

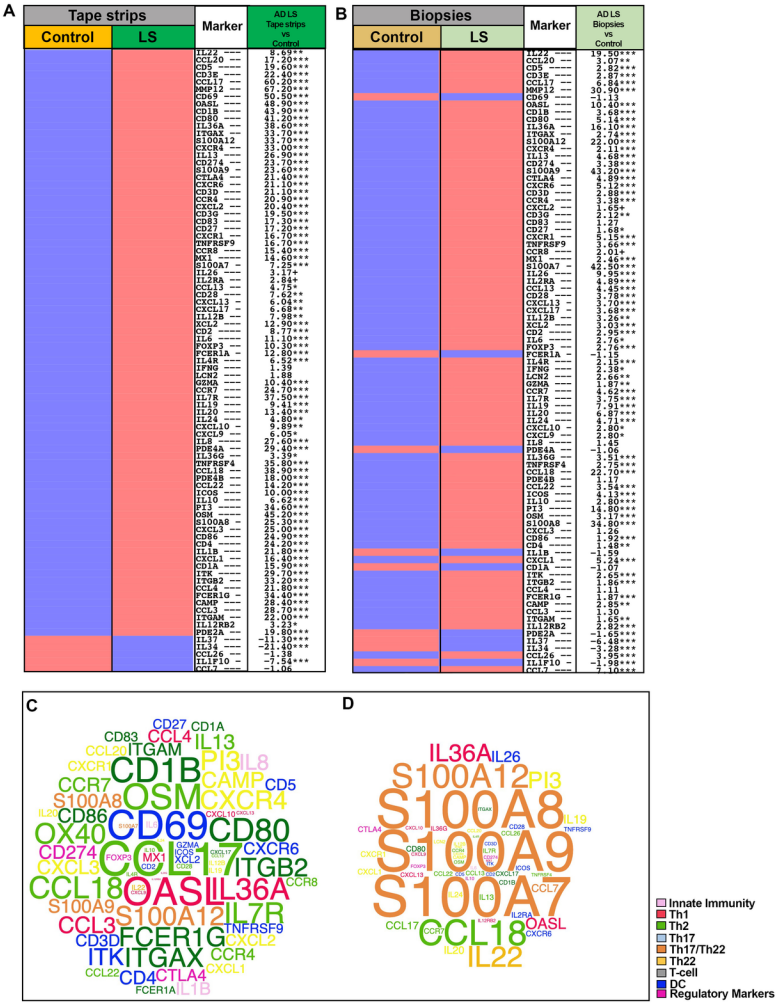

Figure 3

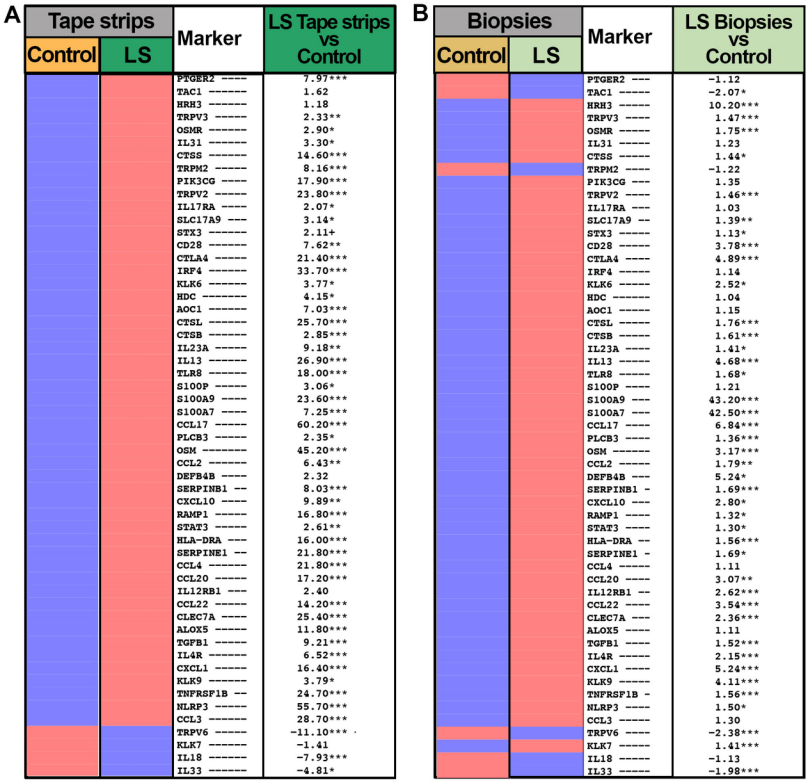

Figure 4

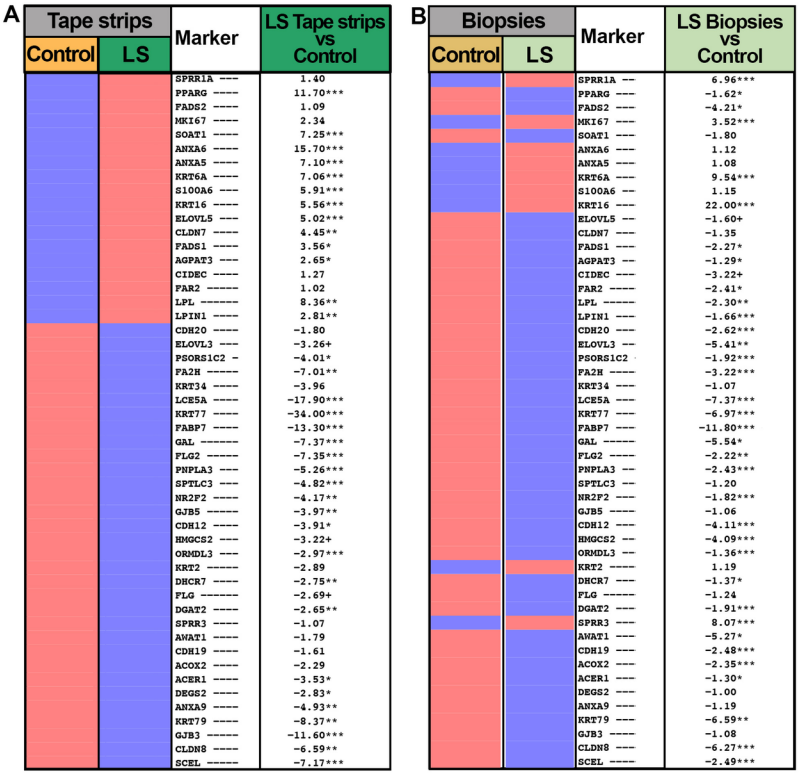

Figure 5