Non-Invasively Stratifying Atopic Dermatitis Patients Based on Inflammatory Genes

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Abstract

Atopic dermatitis (AD) is a chronic inflammatory disease characterized by significant barrier disruption and intense pruritus. In recent years, there has been a growing number of targeted therapies in clinical development with a predominant focus on antagonizing Th2-mediated inflammation; however, these therapies are effective (IGA 0/1) in less than 50% of AD patients. We hypothesized that baseline expression of key inflammatory genes would identify potential subsets of AD patients for a more targeted therapeutic intervention with monoclonal antibody-based therapies. Epidermal skin samples were non-invasively collected from the lesional skin of 31 patients with moderate to severe AD using the 'smart sticker' adhesive skin collection kit . RNA was subsequently isolated and analyzed by RT-PCR for pre-identified genes important to AD disease pathogenesis. IL-4Ra and IL-13Ra1 genes were expressed in 100% of AD patients. Similarly,CCL17/TARC, a biomarker of AD disease severity, was expressed in 96.8% (30/31) of AD patients. Interestingly, the Th2 cytokine IL-13 was expressed in 54.8% (17/31) while IL-31 was expressed in 29.0%(9/31). Additionally, the Th17 associated genes IL-22 and IL-23 were expressed in 51.6% (16/31) and 58.1%(18/31), respectively. Overall, this study demonstrates the potential utility of non-invasive skin sampling to stratify AD patients based on their dominant inflammatory signature and suggests the incorporation of this clinically valuable technique in the personalized treatment of AD patients with targeted therapies.

Methods

Subjects

Thirty-one subjects at least 18 years of age with clinically diagnosed moderate to severe atopic dermatitis and 20 subjects with healthy looking skin (normal skin) were enrolled in this study. Non-invasive skin samples were collected from all enrolled subjects using the DermTech adhesive skin collection kit (DermTech, Inc.; La Jolla, CA) as described below. The study was reviewed and approved by Aspire IRB (Santee, CA). All subjects provided written consent prior to enrollment.

Skin Sampling

The DermTech adhesive skin collection kit (DermTech, La Jolla, CA) was used to collect skin samples from lesional skin and nearby non-lesional skin. Prior to application of the Smart Sticker™ (Figure 1), the target skin was prepped with an alcohol pad to remove oils and then dry wicked with a gauze pad to remove any remaining moisture. Each kit contains a total of 4 Smart Stickers™ for sample collection. Smart Stickers™ were applied individually to the designated area and 5 circular motions with the thumb were used to ensure adhesion to the targeted skin. Using a pen, small marks were made on the skin after placement of the first tape-strip to ensure consistent placement of each subsequent tape-strip. Smart Stickers™ were removed slowly using standard precautions to prevent folding and eliminate potential sources of contamination. Once removed, the Smart Sticker™ was then placed onto the tri-fold sample collector. This process was repeated with the 2nd, 3rd, and 4th Smart Sticker™ on the same lesional/non-lesional skin. Once all 4 Smart Stickers™ were in the tri-fold collector, the collector was carefully folded and placed in a re-sealable bag for overnight shipment (the Smart Sticker™ and tri-fold collector are stable for 10 days at room temperature). Upon arrival at DermTech, samples were stored at -70ºC or colder until RNA extraction.

RNA Extraction and Gene Expression Analysis

RNA was extracted from Smart Stickers™ using a closed-tube, bead-based method. Briefly, Smart Stickers™ were enzymatically digested to extract the genomic material from the adhesive and nucleic acid isolated using magnetic beads on a kingfisher flex instrument (ThermoFisher Scientific). RNA was converted to cDNA for subsequent analysis of genes outlined below using an ABI7900. Cycle threshold (Ct) values of all qPCR reactions were measured and gene expression levels of each target gene were presented as ΔCt (calculated as: ΔCt= Ct (target)-Ct (ACTB, from the same sample)). Differences between normal skin and lesional AD skin ($\Delta\Delta$Ct) were calculated as: $\Delta\Delta$Ct= ΔCt (lesional AD) -ΔCt (normal skin)). Data is presented as normalized gene expression.

Gene	Gene Classification
CCL17/TARC	Disease Severity Marker
IL-4	Th2
IL-4Ra	Th2
IL-13	Th2
IL-13Ra1	Th2
IL-22	Th17/Epidermal Thickening
IL-23A	Th17
IL-31	Th2/Pruritus
TSLP	Pruritus/Eosinophilic

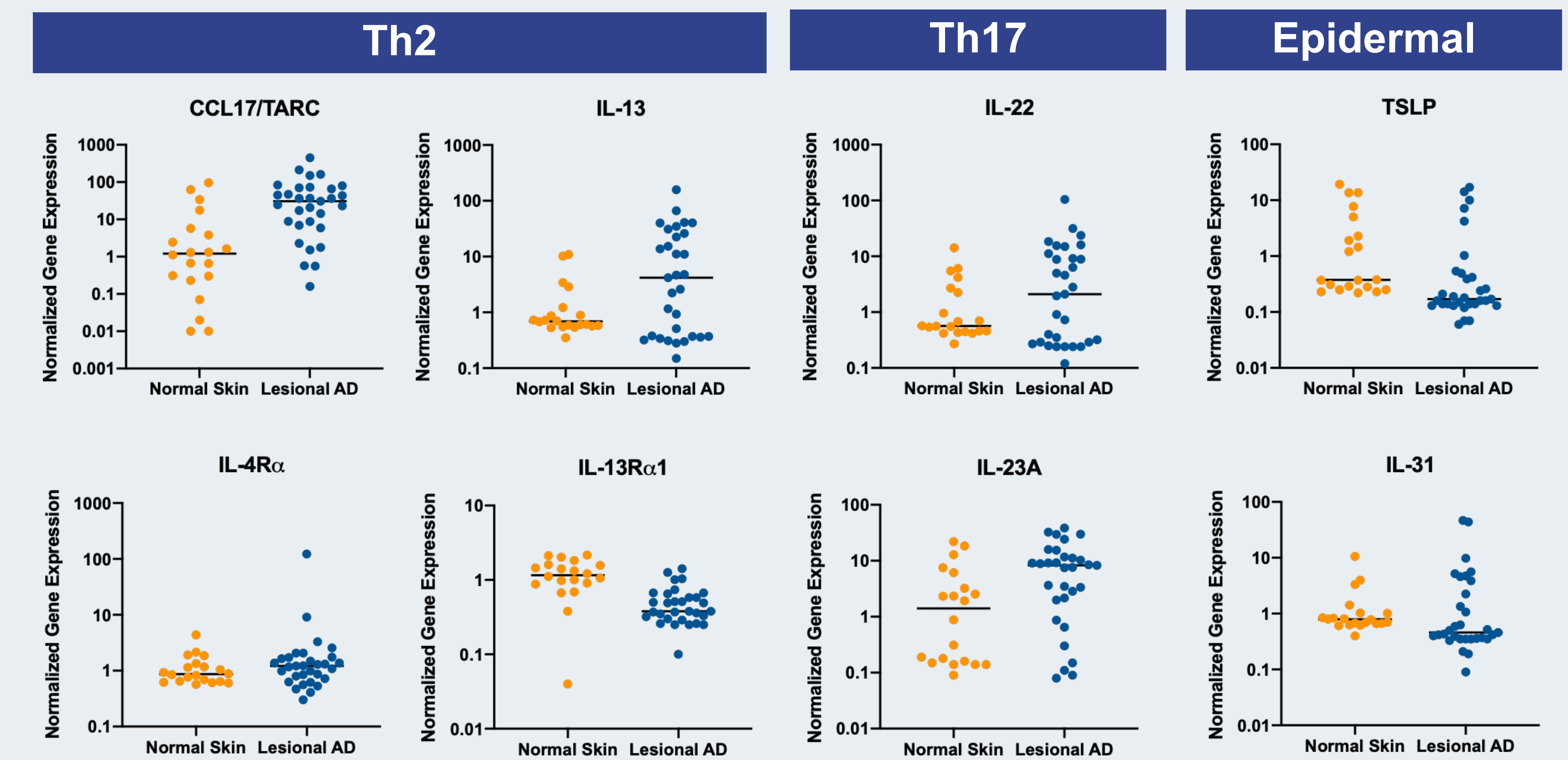## Non-Invasively Assessed Skin Inflammation in Atopic Dermatitis

Figure 1. DermTech Smart Sticker™

*Smart sticker shown with non-invasively collected skin cells*

Non-invasive collection captures genomic and proteomic material in the epidermis

Melanin Granules
Golgi Apparatus
Basal Lamina

PROTEIN | RNA MIGRATION | DNA MUTATION

Illustration of epidermis cross-section

Figure 2. Gene expression of inflammatory biomarkers in non-invasively collected skin samples
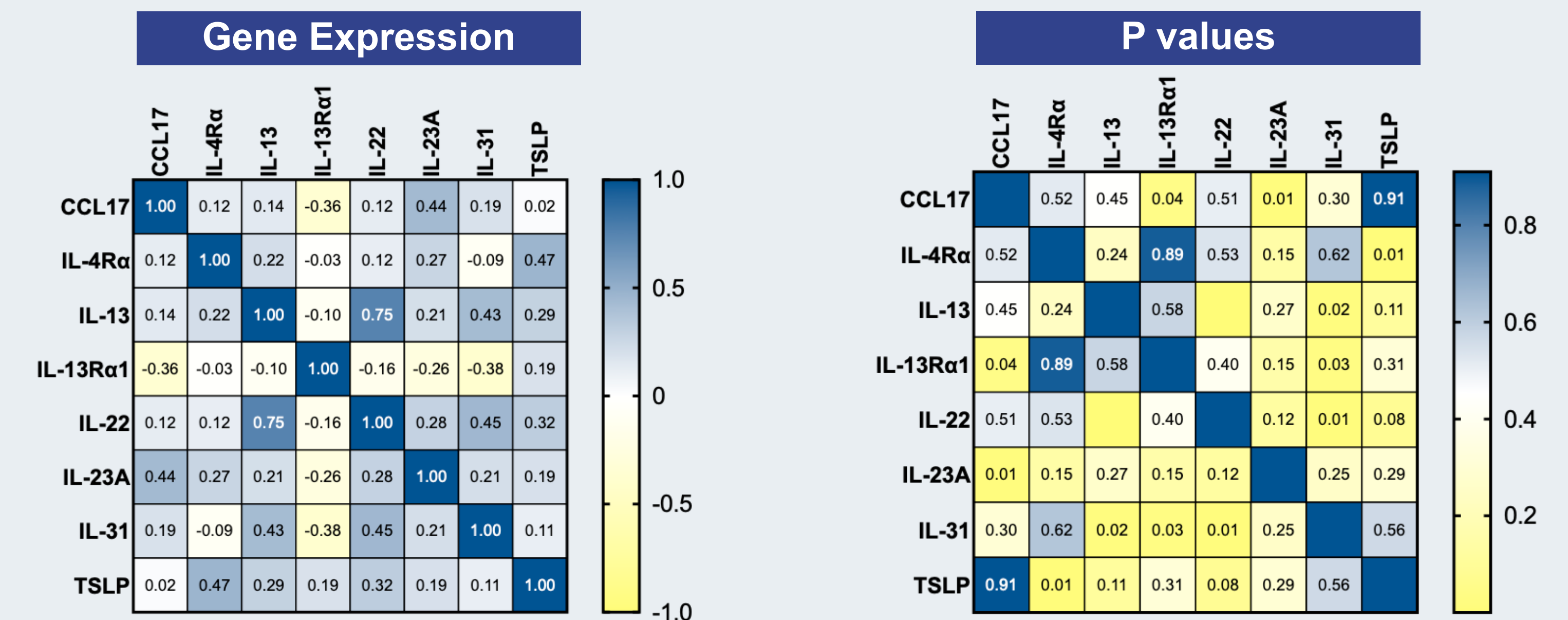

Skin samples were non-invasively collected from normal skin (n=20; orange circles) and lesional AD (n=31; blue circles). Following RNA extraction, inflammatory genes were assessed by RT-PCR. Black bars represent the median value for each group.

Figure 3. Percent of moderate-to-severe AD patients expressing selected inflammatory genes
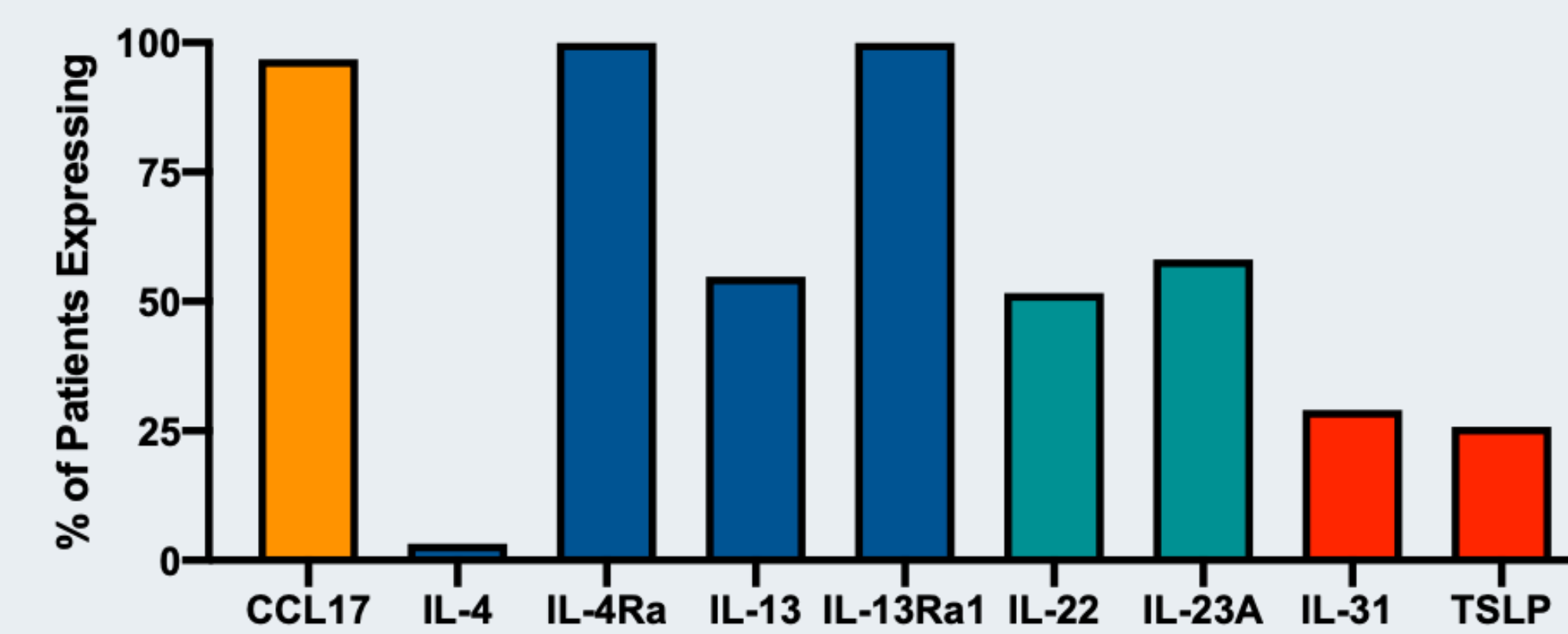
Inflammatory genes were individually evaluated for their expression in lesional AD skin (n=31) to calculate the percentage of subjects expressing each gene. Positive expression was conferred when the cycle threshold was <38.

Figure 4. Heatmap correlation matrix of inflammatory marker gene expression

Gene Expression

	CCL17	IL-4Rα	IL-13	IL-13Rα1	IL-22	IL-23A	IL-31	TSLP
CCL17	1.00	0.12	0.14	-0.36	0.12	0.44	0.19	0.02
IL-4Rα	0.12	1.00	0.22	-0.03	0.12	0.27	-0.09	0.47
IL-13	0.14	0.22	1.00	-0.10	0.75	0.21	0.43	0.29
IL-13Rα1	-0.36	-0.03	-0.10	1.00	-0.16	-0.26	-0.38	0.19
IL-22	0.12	0.12	0.75	-0.16	1.00	0.28	0.45	0.32
IL-23A	0.44	0.27	0.21	-0.26	0.28	1.00	0.21	0.19
IL-31	0.19	-0.09	0.43	-0.38	0.45	0.21	1.00	0.11
TSLP	0.02	0.47	0.29	0.19	0.32	0.19	0.11	1.00

P values

	CCL17	IL-4Rα	IL-13	IL-13Rα1	IL-22	IL-23A	IL-31	TSLP
CCL17		0.52	0.45	0.04	0.51	0.01	0.30	0.91
IL-4Rα	0.52		0.24	0.89	0.53	0.15	0.62	0.01
IL-13	0.45	0.24		0.58		0.27	0.02	0.11
IL-13Rα1	0.04	0.89	0.58		0.40	0.15	0.03	0.31
IL-22	0.51	0.53	0.40			0.12	0.01	0.08
IL-23A	0.01	0.15	0.27	0.15	0.12		0.25	0.29
IL-31	0.30	0.62	0.02	0.03	0.01	0.25		0.56
TSLP	0.91	0.01	0.11	0.31	0.08	0.29	0.56	

Conclusions

- Non-invasive skin sampling with the Smart Sticker™ is a viable method to assess inflammatory signals in lesional AD skin
- Non-invasively assessed inflammatory gene expression has the potential to stratify AD subjects into different subtypes
- Additional studies are needed to evaluate the relationship between baseline inflammation and clinical response to targeted therapies