Feasibility of a novel, non-invasive sample collection technique to develop a molecular test guiding therapeutic selection for patients with atopic dermatitis and psoriasis

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Background

- Recent advances in the understanding of the molecular pathways underlying the development of atopic dermatitis (AD) led to the development of multiple novel systemic drugs targeting those pathways.[1,2]
- As more therapeutics are approved for treatment of AD, it will be important to make informed decisions about each individual patient's therapeutic plan. However, choosing a systemic therapy for AD may not be straightforward.
- Currently approved therapeutics target IL-4 and IL-13 cytokines or the JAK/STAT pathway. Additionally, clinical trials show promise for therapeutic options targeting IL-31, CCR4, Ox40/Ox40L, etc.[1,2,3]
- Further confounding therapeutic selection, a subset of AD can mimic psoriasis. A recent study suggests that clinicians treat these cases empirically more often than consulting pathology.[4] This is likely because biopsies are moderately invasive and can be inconclusive in many of these cases.
- A trial-and-error approach could lead to delay in appropriate treatment of AD or psoriasis and increased cost to healthcare systems.[5] Therefore, understanding individual patient's disease at the molecular level could better inform treatment decisions.
- However, developing a test to incorporate each patient's personal molecular biology into guiding therapeutic selection requires a clinically feasible test.

Objective

- To determine the feasibility of a quick, intuitive, non-invasive skin scraping technique to yield sufficient RNA to assess differentially expressed molecular biomarkers in the epidermis of patients with atopic dermatitis and psoriasis.

Methods

- The superficial epidermis of lesional and non-lesional skin from 20 patients with AD and 20 patients with psoriasis from two dermatology centers in the United States was collected by gently scraping the skin ten times with a curette and immediately preserving in a proprietary buffer.
- Samples were shipped at ambient temperature and frozen at -80 degrees Celsius upon receipt. RNA was isolated, converted to cDNA, pre-amplified, and run on TaqMan OpenArray Real-Time PCR plates to assess relative gene expression of 28 genes by two separate operators.
- For gene expression analysis, the average 2^{-Ct} was compared between lesional and non-lesional skin for AD and psoriasis. A log2 fold change >1 was considered an increase and a log2 fold change <-1 was considered a decrease in gene expression.

Patients
- 20 atopic dermatitis
- 20 psoriasis

→

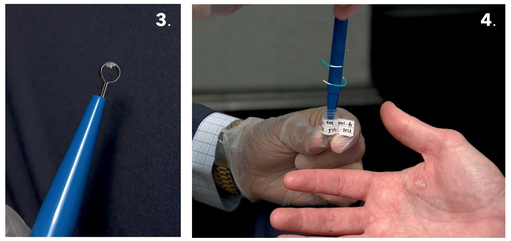
Non-invasive sample collection and preservation
- Lesional skin
- Non-lesional skin

→

Samples Assessed
RNA isolation > cDNA > pre-AMP > qPCR run by 2 different operators > analysis

Methods

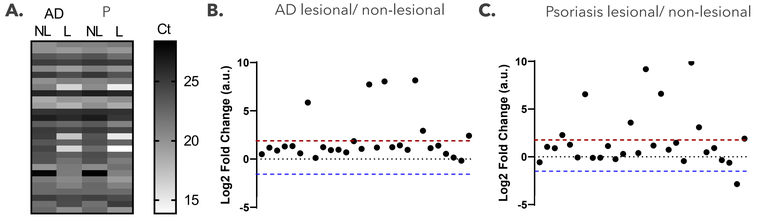
Figure 1. Scraping Technique to Collect Superficial Epidermis Samples



1. Skin prep - cleaning with ethanol swab
2. Gentle scraping with a curette 10 times
3. Quality check
4. Storage in RNA preserving buffer

Results

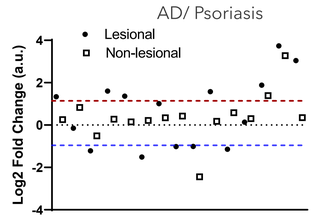
Figure 2. Gene Expression Patterns in Atopic Dermatitis and Psoriasis



A) Raw Ct values of genes assessed for non-lesional (NL) and lesional (L) skin samples from patients with atopic dermatitis (AD) and psoriasis (P). Black indicates higher Ct value (lower gene expression) and white represents lower Ct value (higher gene expression). **B-C**) Log2 fold change of AD (**B**) and psoriasis (**C**) lesional relative to non-lesional samples. Log2 fold change above the red line indicates an increase in gene expression and below the blue line indicates a decrease in gene expression.

Results

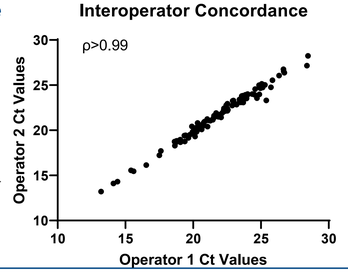
Figure 3. Gene Expression Differences in Atopic Dermatitis and Psoriasis



- Relative gene expression of AD compared to psoriasis for genes that were changed within each respective disease state
- Compared to lesional psoriasis, lesional AD samples exhibited increased expression of seven genes (circles above red line) and decreased expression of three genes (circles below blue line).
- Two of the seven genes with increased expression in lesional AD also demonstrated increased expression in non-lesional AD relative to non-lesional psoriasis samples (squares above red line). One gene had reduced non-lesional expression (square below blue line).

Figure 4. Inter-operator Concordance

- Correlation of average Ct values for each gene by disease state as run by operator 1 compared to operator 2.
- Inter-operator variability was low with a Pearson's correlation of >0.99 observed between operators for average CT values for each gene by disease state.



Conclusion

A non-invasive skin scraping technique produces sufficient RNA to assess reproducible gene expression by quantitative RT-PCR in lesional and non-lesional AD and psoriasis for the purposes of developing a gene expression profile to help guide therapeutic decision-making.

References

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Disclosures

- APQ, MSG, and JW are employees and shareholders of Castle Biosciences, Inc.
- ASF is a consultant for Castle Biosciences, Inc. and on the advisory board for Eli Lilly, Sun Pharma, Orthodermatologics, Boehringer Ingelheim, Incyte, Amgen, Galderma, Novartis and Pfizer.
- JIS is a consultant and/or advisor for Abbvie, Afyx, Aobiome, Arena, Asana, Aslan, BioMX, Biosion, Bluefin, Bodewell, Boehringer-Ingelheim, Cara, Castle Biosciences, Celgene, Connect Biopharma, Dermavant, Dermira, Dermtech, Eli Lilly, Galderma, GlaxoSmithKline, Incyte, Kiniksa, Leo Pharma, Luna, Menlo, Novartis, Optum, Pfizer, RAPT, Regeneron, Sanofi-Genzyme, Shaperon, Sidekick Health; speaker for Abbvie, Eli Lilly, Leo Pharma, Pfizer, Regeneron, Sanofi-Genzyme; institution received grants from Galderma, Pfizer