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Characterization of Inflammatory Cytokine Gene Expression in a Family with a History of Psoriasis

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Summary

Psoriasis is a heritable autoimmune disorder characterized by abnormal red and itchy skin patches. To determine if the children of a father with psoriasis express psoriasis-related biomarkers without demonstrating clinical signs of the disease, epidermal skin from the mastoid process behind the ear was harvested from four family members, RNA was extracted from it, and the expression of ten genes involved in the inflammatory cytokine pathway of psoriasis were analyzed by qRT-PCR. Gene expression for TNF-α, a cytokine known to cause psoriasis, was found to be consistent with the family members' disease status; while the father showed elevation of TNF-α gene expression, TNF-α was not expressed in other family members. No other inflammatory cytokines associated with psoriasis were found to be significantly elevated including in the mother, who is disease-free and served as a control. Antimicrobial peptides and other markers of active psoriatic disease were not elevated in the father or other family members. Elevations in lymphocyte migration factor and IL-23a found in one child could be explored as potential predisposing factors to future disease development.

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Introduction

Psoriasis is an autoimmune inflammatory disease characterized by abnormal skin patches that are typically red, itchy, and scaly, referred to as plaques (1). Normal skin cells have a cycle of about 25–28 days, when the skin cells gradually shift up and flake off. The cell cycle in the skin cells affected by the rapid proliferation of keratinocytes is shortened to about 3–6 days, causing the buildup of scaly red plaques (1). This abnormal skin can form anywhere on the body and is primarily mediated by white blood cells that migrate to the epidermis and secrete different cytokines, mainly TNF-α, IL-17, and IL-23A (2). Once these cytokines are released, they trigger the rapid proliferation and differentiation of keratinocytes in the epidermis. In addition, the keratinocytes also secrete proteins that attract more white blood cells, resulting in a vicious cycle of excessive inflammation (1).

Psoriasis is thought to be a heritable disease that can be triggered by environmental factors (2). There are several genes linked to psoriasis, such as those in the PSORS gene family (PSORS1-PSORS9) (3). The skin of patients with psoriasis can express cytokine and inflammation markers in areas that are unaffected by plaques, and cleared areas of skin can have a "molecular scar" of inflammatory markers (4).

Given the heritable nature of the disease, we studied a family in which the father had been diagnosed with psoriasis. We were interested in understanding if the children, who did not show any symptoms, would demonstrate gene expression consistent with the disease. We used the mother of the family, who was negative for the disease, as a control. To conduct this work, we collected samples using a non-invasive adhesive patch epidermal biopsy kit and measured gene expression of key psoriasis inflammatory makers using quantitative RT-PCR (qRT-PCR). Ten genes involved in the inflammatory cytokine pathway of psoriasis were analyzed by qRT-PCR. Only the father had documented psoriatic disease and showed elevation of TNF-α gene expression, as compared to the children. Other inflammatory cytokines (IL-23, IL-17, and IL-22) were not elevated in the father or any of the other family members.

Results

Only the father had documented psoriatic disease. An average of 1,813 pg of RNA (s.d. 2325 pg) was isolated from adhesive patch biopsy samples, as quantified by beta-actin. The father showed elevation of TNF-α gene expression, a cytokine known to cause psoriasis, which was not present in either child or the mother. The inflammatory cytokines IL-23, IL-17, and IL-22 were not elevated in the father or any of the other family members. There were no discernible differences in the pattern of expression of lymphocyte adhesion and migration biomarkers between either the father and the children or the mother and the children, though one child

Subject Initials	Relation	Age	Gender	Psoriasis Status
1	Father	51	Male	Yes
2	Mother	47	Female	No
3	Child	16	Male	No
4	Child	12	Male	No

Table 1: Characteristics of enrolled subjects.

exhibited slightly elevated levels of IL-23A and CCL20. Antimicrobial peptides and other markers of active psoriatic disease were not elevated in the father or other family members.

There was a total of 4 subjects from a single family enrolled in the study: 3 males and 1 female, with age ranges between 12 and 51 (**Table 1**). The only subject with psoriasis was the father.

A non-invasive epidermal skin biopsy kit and procedure was used to collect the samples in this study. An average of 1,813 pg of RNA (s.d. 2325 pg) was isolated from adhesive patch biopsy samples, as quantified by comparing the number of beta-actin transcripts to human reference RNA (**Figure 1**). The mother (Subject 2) had a significantly higher RNA yield compared to the other three subjects. There were no differences between subjects in control gene beta-actin cycle counts, indicating an equivalent amount of RNA input was used in each reaction and allowing for comparisons of each subject based on raw cycle counts without normalization against beta-actin (**Figure 2**).

Figure 3 shows the detected Ct values of some of the major cytokines involved in psoriasis. There were no differences between IL-17F, IL-22, and IL-23A. However, there was significantly higher (32-fold) expression of TNF-α for the father, which was not seen in any of the other subjects. TNF-α is a cytokine known to stimulate

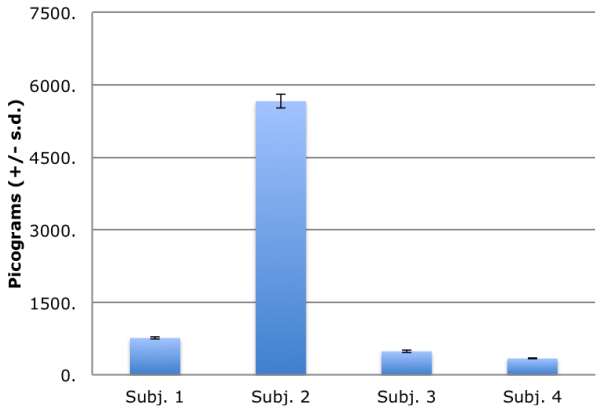

Figure 1: Total RNA Yield from Stratum Corneum Adhesive Biopsy Samples. Subject 1 has psoriasis; Subject 2 has no disease or predisposition, Subject 3 has no disease but predisposition, Subject 4 has no disease but predisposition.

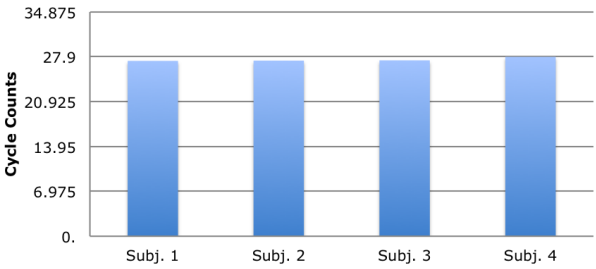

Figure 2: Cycle counts of the beta actin control gene associated with 15 pg of total RNA added to the reverse transcription reaction. The uniformity of the cycle counts across subjects indicates that nearly identical quantities of RNA were assessed, allowing the analysis of actual cycle counts for each gene.

formation of psoriatic lesions. had a slight increase in IL-23A gene expression.

Antimicrobial peptide (AMP) expression by keratinocytes is stimulated by inflammatory cytokines, and their levels can be elevated in psoriatic lesions (5). This study did not show any differences in AMP expression in non-lesional skin between family members and Subject 1, the known psoriasis carrier (**Figure 4**). There were also no differences between family members in the expression of CXCL1 and CXCL5, chemokines that are secreted by keratinocytes (**Figure 5).** However, OD showed a slightly higher expression of CCL20.

Discussion

Farinas et al. studied the skin of psoriatic patients for gene expression and found that uninvolved skin displayed evidence of inflammation similar to involved skin (4). Even though OD and Subject 3 showed no signs of psoriasis, we hypothesized that their skin might similarly show changes in gene expression consistent with psoriasis since the father has the disease and it is heritable. Four family members: the mother, two brothers, and the father were evaluated. Looking at the major cytokines tested, there appeared to be no discernable difference between the subjects for IL-17F, IL-22, and IL-23A expression. However, the father had a significantly higher expression level of TNF-α, which is a major cytokine in the formation of psoriasis lesions. There appeared to be no differences between family members for anti-microbial peptides and chemokines that are primarily expressed by keratinocytes in the skin. Looking at the anti-microbial peptides, hardly any changes between the subjects were observed. This is most likely due to the fact that these peptides are elevated in lesional skin, whereas the skin we tested was non-lesional, which has a much lower level of target gene expression (5). One exception is Subject 4, who showed higher levels of CCL20, a chemokine, and IL-

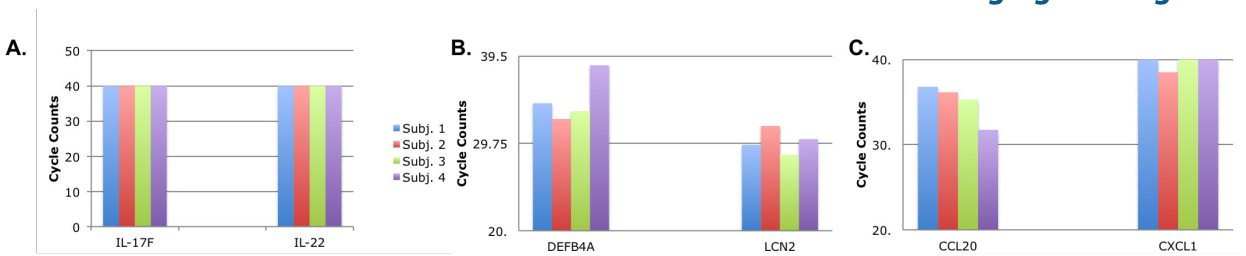

Figure 3. A. Gene expression of inflammatory cytokines. Cycle count values of 40 were imputed for reactions that did not have any transcripts detected. **B.** Antimicrobial peptide gene expression from each subject. **C.** Gene expression levels of chemokines involved in migration of leukocytes to the skin.

23A, an inflammatory cytokine known to significantly contribute to psoriasis. It is possible that this expression pattern could be predictive for developing psoriasis in the future, and it will be interesting to observe this subject's skin over time. While we only tested 4 subjects in a single family and a small subset of the genes related to psoriasis expression, additional subjects and genes would provide more data and allow for more definitive conclusions.

Methods

Study Subjects and Skin Sample Collection

The skin samples were collected from 4 family members: Subject 4 (12 years old) and Subject 3 (16 years old), brothers, who do not have the disease but have a predisposition, Subject 3 (16 years old), Subject 2 (47 years old), the mother, who does not have the disease and does not have any predisposition, and Subject 1 (51 years old), the father, who has the disease. All of the samples were extracted from the mastoid area of the subjects, while all of the sampled skin was non-lesional for the purpose of comparison. Subject 1 is a known psoriasis carrier while the other three subjects show no symptoms (**Table 1**). Skin samples were obtained according to the instructions for use for the DermTech adhesive biopsy kit (**Figure 6**). Before taping, the target area was cleaned with an alcohol wipe and dried with a gauze pad. The adhesive patches were firmly placed and rubbed on the target area and then slowly removed. After every patch, the area was wiped with a gauze pad to remove any secretions from the skin. Four patches were used to collect one sample from each area.

Gene Expression Analysis

Total RNA was isolated from the stratum corneal tissue on the adhesive patches using a modified PicoPure™ procedure (Life Technologies, Foster City, CA), and reverse transcribed to complementary DNA (cDNA) using SuperScript® VILO™ cDNA Synthesis Kits. The resulting cDNA was subsequently used for target gene expression analysis with qRT-PCR on an ABI7900 PCR system (Life Technologies). Total RNA was quantified using the beta-actin gene and a standard curve for beta-actin created from serial dilutions of Universal Human Reference RNA. Each qRT-PCR reaction used 15pg of total RNA, in duplicate in 20uL volume on 384-well PCR reaction plates using pre-designed gene-specific TaqMan probe chemistries (Life Technologies). An averaged cycle threshold (Ct) value of the duplicate measurements was used in the analysis. Gene expression was determined for the following

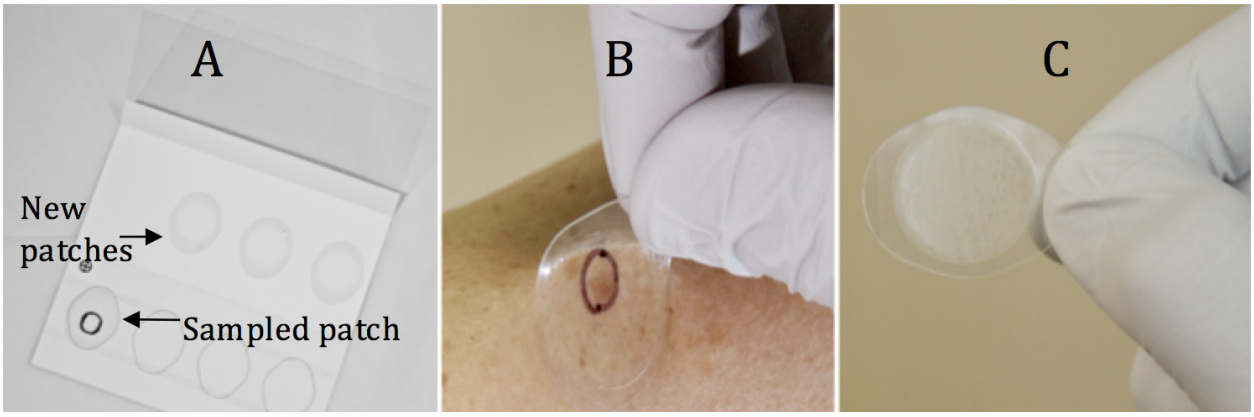

Figure 6: Adhesive skin sample collection kit. A. trifold with adhesive patches; **B.** epidermal collection; **C.**epidermal cells on an adhesive patch.

genes: chemokine (c-x-c motif) ligand 1 (CXCL1), chemokine (c-c motif) ligand 20 (CCL20), beta defensing 4A (DEFB4A), lipocalin 2 (LCN2), S100 calcium binding protein A9 (S100A9), tumor necrosis factor alpha (TNF-α), interleukin 17 F chain (IL-17F), interleukin 22 (IL-22), and interleukin 23 sub-unit alpha (IL-23A). All genes with a measurable Ct value were confirmed by reviewing the amplification curve. For reactions that yielded an 'undetermined' Ct value (amplification curve never above detection threshold) the Ct value was imputed as 40. In addition to the target genes, human beta-Actin was used as an internal control.

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