Detection of IL-36γ through noninvasive tape stripping reliably discriminates psoriasis from atopic eczema

To the Editor:

Inflammatory skin reactions, regardless of their distinct underlying pathophysiologic mechanisms, can often present with similar morphology. Although typical eczema and lesions of plaque psoriasis are easily distinguishable by experienced dermatologists, these 2 diseases often cause diagnostic difficulties when inflammation is minimal or located in certain anatomical regions, including flexures, the scalp, auricular or palmoplantar areas. In these cases diagnosis can be problematic, in particular in the primary care setting, where misdiagnosis can lead to delayed appropriate treatment, overuse of antibiotics, or both.

Histopathology, which is the current gold standard in diagnosing challenging cases, is invasive, costly, and often unavailable in the primary care setting. Furthermore, histopathologic differentiation of psoriasis from eczema in some anatomical localizations, such as the palmoplantar region, is difficult. Unfortunately, there are also no reliable blood-derived diagnostic biomarkers that cover the wide range of clinical phenotypes and disease severities, and although RNA signatures from lesional skin biopsy specimens have been described for atopic dermatitis (AD) and psoriasis, mRNA analysis can be costly and labor intensive. Therefore a simple, noninvasive, and reliable diagnostic approach would be of great clinical benefit. To address this need, this study uses a noninvasive, tape-stripping, and ELISA-based approach to investigate potential protein biomarkers that are able to discriminate eczematous from psoriatic inflammation. This study presents a range of severities.

Because the epidermis is a significant source of chemokines, inflammatory lesions from patients with psoriasis and those with AD were sampled initially by means of tape stripping and analyzed for the neutrophil-recruiting chemokines CXCL1 and IL-8, as well as CCL20, which recruits IL-17/IL-22-producing cells (see the Methods section in this article’s Repository at www.jacionline.org for detailed methodology). Although these chemokines were found at significantly greater levels in tape-stripping samples from lesions of patients with psoriasis compared with those with AD, receiver operating characteristic (ROC) curve analysis indicated that they would not be ideal as strong discriminators of the 2 conditions (IL-8: area under the curve [AUC], 0.83; SE, 0.0523; 95% CI, 0.726-0.931; CXCL1: AUC, 0.796; SE, 0.049; 95% CI, 0.70-0.891 [Fig 1, B]; CCL20: AUC, 0.82; SE, 0.045; 95% CI, 0.731-0.905). For all 3 chemokines, no or very low levels of protein were detected in healthy and nonlesional samples.

Because high levels of IL-36γ mRNA and protein have been reported in lesions of patients with psoriasis, IL-36γ from tape samples was quantitated through use of a novel in-house sandwich ELISA (see the Methods section in this article’s Online Repository). IL-36γ showed a trend for increased levels in patients with AD compared with levels seen in nonlesional or healthy skin (mean, 71/13.5/57.5 pg/μg total protein, respectively). However, IL-36γ levels within psoriatic lesions were significantly greater than those in AD lesions (mean, 719 vs 71 pg/μg total protein; Fig 1, A). No difference in IL-36γ expression was observed in healthy versus nonlesional psoriatic skin taken from ventral forearm areas.

A ROC curve (AUC, 0.987; SE, 0.0114; 95% CI, 0.965-1.01) was plotted to determine the sensitivity and specificity of IL-36γ in a diagnostic approach (Fig 1, A). At an optimal cutoff level of 214 pg/μg total protein (Youden index, 0.944), IL-36γ specificity was calculated as 100% (95% CI, 90% to 100%), and sensitivity was calculated as 94.44% (95% CI, 84.61% to 98.84%). This suggests that IL-36γ has an excellent potential as a diagnostic marker for psoriatic inflammation. When compared with CXCL1, the increased expression of IL-36γ is far more consistent, being found across all patients with psoriasis. We also measured IL-36γ expression in a range of other skin pathologies, including fungal infection, lichen planus, systemic-, subacute cutaneous-, and chronic discoid lupus erythematosus, all of which showed expression levels less than the cutoff level of 214 pg/μg total protein (data not shown).

Both CCL27 and CCL17 have been suggested as potential biomarkers for AD. CCL17 preferentially attracts IL-4/IL-13–producing lymphocytes, whereas CCL27 is involved in the homing of memory T cells to the skin. Epidermal tape stripping demonstrated CCL17, but not CCL27, to be of value in identifying AD (Fig 1, C). CCL27 levels were increased in both psoriatic and AD lesions. In those cases in which CCL17 was detectable, it pointed to an underlying AD inflammation (AUC, 0.79; SE, 0.058; 95% CI, 0.676-0.903). However, unlike IL-36γ, which was detectable in all psoriatic lesions tested, a significant number of AD samples (25%), as well as 57.4% of psoriatic samples, did not show any measurable CCL17.

The patient cohort (see Table E1 in this article’s Online Repository at www.jacionline.org) included in our analysis showed prototypic plaque psoriasis or AD. However, to further illustrate the potential of tape-collected IL-36γ as a diagnostic approach for psoriasis, we investigated clinical cases of unclear diagnosis. In each case the diagnostic value of IL-36γ was correct when including dermatohistopathologic results. As an example, we show 2 clinical cases (for details, see the Methods section in this article’s Online Repository).

The first case was given a clinical diagnosis of and treated for palmoplantar eczema in our dermatology department. However, analysis of tape stripping showed IL-36γ levels of 960 pg/μg total protein, which are indicative of psoriasis. This fact and subsequent dermatohistopathologic analysis and changes in clinical features were supportive of a corrected diagnosis to that of psoriasis (Fig 2, A).

The other case was a patient with symptoms of joint pain in addition to erythrosquamous skin lesions. For the referring rheumatologist, a confirmation of these skin lesions as psoriasis would guide future diagnostic and treatment pathways in the direction of psoriatic arthritis. However, tape stripping did not confirm psoriatic inflammation, and the diagnosis for the skin lesions was confirmed to be chronic eczema (Fig 2, B).

Numerous soluble mediators, cell-surface molecules, and intracellular proteins have been described previously to be upregulated in psoriatic lesions. In our noninvasive approach we could confirm that levels of the neutrophil chemoattractants IL-8 and CXCL1, as well as CCL20, the chemoattractant for IL-17–producing cells, are increased in psoriatic compared with
eczema lesions. Contrary to work showing reduced expression of CCL27 in patients with psoriasis and the value of CCL27 as a biomarker for AD, we did not find significant differences in the amount of this chemokine. The differences between our findings and those of some others is likely to be due to the method of sampling.

In conclusion, although the effects of other factors, such as systemic and topical therapeutics, age, sun exposure, and lesion chronicity, need to be investigated in future studies, the results presented here confirm IL-36γ to be a robust, specific, and reliable biomarker for psoriatic inflammation that outperforms previously reported biomarkers and is likely to withstand all challenges in real-life primary and secondary dermatologic care.

We thank the patients and volunteers who agreed to participate in the study. We especially thank Jennifer Ott, Fiazia Ahmed, Rebecca Johnston, and Julie Bush for patient identification and clinical trial management.
FIG 2. Tape stripping for the diagnosis of difficult clinical cases. Shown are pictures of 2 clinical presentations in which the clinical diagnosis was unclear or challenging. Based on an IL-36γ cutoff level of 214 pg/μg total protein, tape stripping results indicated psoriatic inflammation for case A but not case B. Histopathologic examination confirmed the results. A, Palmar psoriasis was misdiagnosed initially as hand eczema. B, Chronic eczema. For further details on Fig 2, see this article’s Online Repository at www.jacionline.org.
Association of ST2 polymorphisms with atopy, asthma, and leukemia

To the Editor:

The phenome-wide association study (PheWAS) is a new, validated, reverse genetics approach that associates genetic variants of interest with phenotypes by linking a database of deidentified genotyping to a broad range of electronic medical record–derived clinical phenotypes.1 Although the more common genome-wide association study (GWAS) is useful in identifying genetic polymorphisms that may relate to one particular disease phenotype, PheWAS’s ability to evaluate these polymorphisms against an entire database of clinical phenotypes greatly expands the scope of genomic association research. Using a PheWAS, one is able to examine polymorphisms detected in the context of a disease to not only confirm this established association with greater certainty, but one may uncover links to entirely new phenotypes that had not been previously considered or investigated at the genetic level.1

IL-33 is one of the most consistently associated gene candidates for asthma identified by GWASs in diverse ethnic groups.2–4 IL-33 is a central mediator of both innate- and adaptive immunity–regulated allergic inflammation in the lung.2,3 The primary binding partner of IL-33, ST2, is a member of the IL-1 receptor family that is expressed on a number of immune cells, including ILC2, CD4+ Treg cells, mast cells, and eosinophils.3 GWASs investigating polymorphisms related to asthma have identified a number of single nucleotide polymorphisms (SNPs) in the ST2 receptor gene.5–7 We sought to investigate these polymorphisms to assess the role of ST2 signaling in other, non–asthma-related allergic diseases. In addition, we hoped to identify new diseases in which genetic links to the ST2 receptor had not been reported. We performed a PheWAS on 15 ST2 genetic variants implicated in asthma to assess their associations with other allergic and nonallergic diseases. The Exome BeadChip that we used to perform the PheWAS genotyped 38 variants in ILIRI. We only included variants with a minor allele threshold (mean allele frequency [MAF]) of greater than 1%; this filtering is necessary to support a PheWAS analysis using a logistic regression. The PheWAS used a cohort of 36,400 adult (age > 18 years) subjects who had genotyping on Illumina HumanExome BeadChip version 1.1 and available electronic health record (EHR) data from the Vanderbilt BioVU DNA biobank that was ascertained previously for 5 different criteria: (1) eligibility as case or control in 1 of 31 pharmacogenetic studies, (2) available longitudinal data with primary care visits, (3) presence in the cancer registry, (4) old age with longitudinal data, and (5) presence of rare diseases or conditions ascertained via billing codes. Of the 15 SNPs, 6 had MAFs greater than 1% in the total cohort population, rendering them sufficiently powered for further analysis.1 All these 6 SNPs are in the ST2 gene (human chromosome 2q11.2).6 The PheWAS yields results as a list of phenotype International Classification of Diseases, Ninth Revision (ICD-9) codes that best associated with each particular SNP.

Our study revealed that the minor allele of ST2 SNP rs1041973 associated with allergic conjunctivitis (odds ratio [OR] = 1.27), an allergic disease that had not been linked genetically in the context of IL-33/ST2 signaling. We further demonstrate that the minor allele of rs1041973 exhibits an inverse association with eosinophilia, as indicated by an OR of less than 1 (0.686; Table I). In addition, the PheWAS confirmed the previously reported inverse relationship of 5 ST2 SNPs with asthma (rs10192036: OR = 0.876; rs1041973: OR = 0.505; rs4988956: OR = 0.876; rs10192157: OR = 0.876; rs10206753: OR = 0.876), and it confirmed the formerly identified association of rs1420101 (OR = 1.20) with asthma.6,7 We calculated the pair-wise linkage disequilibrium for each of the 6 variants tested and found that the minor alleles of rs10192036, rs10206753, rs4988956, and rs10192157 are in perfect linkage (r2 = 1). For this reason, the associations of these 4 SNPs are reported under only rs4988956. These genomic investigations with study populations entirely separate from our own provide ORs in similar directions for all 6 SNPs investigated in this study, confirming the previously described association of each.6,7 This further supports the validity of our method by demonstrating that genetic and phenotypic data from Vanderbilt University Medical Center patients is representative of national and international populations.6,8 Finally, the PheWAS uncovered a novel association of 2 SNPs in the ST2 gene with various forms of leukemia (Table II). The minor allele of the SNP rs1420101 associated with leukemia and acute myeloid leukemia, whereas the minor allele of rs1041973 associated with leukemia, acute myeloid leukemia, and acute lymphoid leukemia.

The results of this study inform our understanding of the ST2 pathway as it relates to other, nonasthmatic forms of allergic disease. We are also the first to identify rs1041973 as a genetic variant that associates inversely with eosinophilia. In an analysis of peripheral blood eosinophilia for eosinophilia-associated rs1041973 cases and controls, there was a significant increase in the maximum-recorded percentage of peripheral blood eosinophils in cases (13.5%) compared with controls (3.4%; P = 3.15 × 10−165, see Table E1 in this article’s Online Repository at www.jacionline.org). Because SNPs examined in this study were originally identified in a GWAS as relevant

REFERENCES


METHODS

Patients
Patients with a clinical diagnosis of plaque psoriasis or AD presenting with “typical” morphology, as diagnosed by consultant dermatologists, were included in the study. Hanifin and Rajka diagnostic criteria were followed for eczema, and diagnosis of plaque psoriasis was based on traditional pattern recognition combined with patient history. Patients’ demographics are shown in Table E1.

This study was approved by NRES Committee North East-York (ILTIPP study: REC 14/NE/1199, ALPHA study: REC 14/YH/1259) and for recruitment of volunteers by the ethics committee of the University of Leeds (BIOSCI 14-001). All participants provided written informed consent before participation.

Overall disease activity differed significantly and was not collected for analysis because the focus was on the individual lesion. A local lesion severity score was assessed based on Psoriasis Activity and Severity Index items (covering the range of 0-4) by specialist dermatologists before sample collection (see Table E2). Patients receiving systemic immunomodulatory therapy or biologics were excluded apart from 1 patient receiving anti–IL-36γ antibody generated by rat hybridoma HCL17 performed effectively as a capture antibody, and the mouse hybridoma B5A2 performed effectively as a detection antibody by using sandwich ELISA. Antibodies were subsequently purified with either protein A or protein G affinity chromatography. The antibody HCL17 was then biotinylated with EZ link NHS-LC-biotin (Thermo Scientific, Waltham, Mass), according to the manufacturer’s instructions.

ELISA
Immunosorbent 96-well ELISA plates (Nunc Life Technologies, Paisley, United Kingdom) were coated with 2 μg/mL BSA capture antibody in PBS at 4°C overnight. Plates were then washed 3 times with 0.1% Tween 20/PBS and blocked for 1 hour in 2% BSA in 0.1% Tween-20/PBS. Samples were incubated subsequently for 1 hour at room temperature before washing and incubation with 1 μg/mL HCL17 biotinylated detection antibody for 1 hour. Plates were then washed and incubated with streptavidin–horseradish peroxidase (BioLegend, London, United Kingdom) for 20 minutes. After washing, TMB was used as a chromogenic substrate (Thermo Scientific). The reaction was stopped with 2N H2SO4, and OD was measured at 450 nm. A standard curve was obtained from a 7-point serial dilution of protein standard and used to calculate IL-36γ concentrations. CCL27 from the tape-stripping samples was quantified by means of ELISA (Bio-Technne, Abingdon, United Kingdom), according to the manufacturer’s protocol.

Multiplex bead-based quantification assays
Cytokines (CCL20, CXCL1, and IL-8) from tape-stripping samples were measured by using bead-based immunoassay purchased from BioLegend. The assay was carried out according to the manufacturer’s instructions, and fluorescent beads were acquired by using flow cytometry (LSRII; BD Biosciences). The results were analyzed with LEGENDplex analysis software (BioLegend).

Histopathology
Routine diagnostic punch biopsy specimens were taken from lesional skin, fixed in 10% formaldehyde, embedded in paraffin, stained with hematoxylin and eosin, and evaluated by experienced dermatopathologists.

Statistical analysis
Results were analyzed with GraphPad Prism software, version 7.00 (GraphPad Software, La Jolla, Calif). Results for each group are depicted as means ± SDs. Data were analyzed by using 1-way ANOVA followed by the Tukey multiple comparison test to determine statistically significant differences between groups. Sensitivity and specificity analysis was performed by using ROC curve analysis. The optimal cutoff level was determined according to the Youden index.

Clinical cases
Case A, a 61-year-old male patient, received a diagnosis of chronic palmoplantar eczema based on clinical appearance and symptoms (Fig 2, A). There were no signs of eczema or psoriasis elsewhere on the body. The patient had no personal history of atopy; family history was positive for allergic asthma and negative for psoriasis. Patch testing revealed a positive reaction to methylchloroisothiazolinone, exposure to which the patient avoided subsequently. The patient’s total IgE level was 767 kU/L without detectable specific IgE to inhaled allergen mix. Mycology microscopy and culture results were negative for fungal infection. The patient showed very little to no benefit from immersion PUVA therapy and presented little response to 30 mg/d oral alitretinoin. During the disease course, lesions gained a slightly different appearance as coarser scales developed. Tape-stripping samples were taken before a diagnostic biopsy was obtained from a right palmar lesion. Results were highly suggestive of the diagnosis of psoriasis, with significantly enhanced levels of IL-36γ (IL-36γ, 959.3 pg/μg; CXCL1, 15.9 pg/μg; and IL-8, 29.2 pg/μg; CCL17 was not detected). Histopathologic examination from the same site confirmed the diagnosis of psoriasis. Subsequently,
re-examination of the patient revealed some nail pitting (Fig 2, A), a symptom associated with psoriatic inflammation.

Case B, who was referred by the rheumatology department, presented with unclassified polyarthritis and some skin symptoms. The presence of relatively well circumscribed margins and a thick adherent scale included psoriasis as a differential diagnosis (Fig 2, B). However, low expression of IL-36γ in tape-stripping samples did not suggest psoriasis (IL-36γ, 25.2 pg/µg; CXCL1, 0.12 pg/µg; and IL-8, 0.25 pg/µg; CCL17 levels were less than detection levels). Subsequent histopathologic examination confirmed the diagnosis of eczema.

Detailed legend of Fig 2

A, **Upper pictures**, Typical clinical signs of eczema: palmar erythema with moderate infiltration of the skin, deeply seated vesicles with discrete erosions, and fine scaling associated with intense itchiness. **Lower pictures**, The same patient 3 months later presented a change in clinical phenotype with the presence of rare intact pustules accompanied by accentuated scaling and typical nail changes showing pitting and ridging. Histopathologic examination shows regular epidermal hyperplasia, with clubbing and anastomosis of the elongated rete ridges associated with suprapapillary plate thinning. Mild, patchy, perivascular infiltrate composed of lymphocytes without eosinophils and mild exocytosis is present. The granular layer is still present as well. However, there is some parakeratosis in which collections of neutrophils are identified (hematoxylin and eosin stain; high-power magnification ×400). B, **Upper pictures**, Relatively well-demarcated, slightly erythematous, intensely itchy lesion covered by thick adherent scales in the left lateral supramalleolar localization of a 51-year-old female patient. Histopathologic examination showed irregular epidermal hyperplasia in association with hyperkeratosis and hypergranulosis. Patchy epidermal spongiosis with focal lymphocytic exocytosis can be seen. There is no club-shaped elongation of the rete ridges, and neutrophils are not identified. The features are in favor of those of a lichenified eczema (hematoxylin and eosin stain; high-power magnification ×400).

REFERENCES


**TABLE E1. Demographic characteristics of patients**

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<th>Patients with AD</th>
<th>Patients with psoriasis</th>
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<tr>
<td>Sex</td>
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<tr>
<td>Male</td>
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<td>Age (y)</td>
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<tr>
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<tr>
<td>Range</td>
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TABLE E2. Comparison of local severity scores of patients with AD and psoriasis

<table>
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<th></th>
<th>Patients with AD</th>
<th>Patients with psoriasis</th>
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<tr>
<td>Mean erythema score</td>
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<td>Mean infiltration score</td>
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<td>Mean scaling score</td>
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<tr>
<td>Mean total lesion score</td>
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A local severity score was determined by experienced dermatologists for all patients with eczema and psoriasis included in the study.