

Letter to the Editor

Peeling off the layers: Skin taping and a novel proteomics approach to study atopic dermatitis*To the Editor:*

Atopic dermatitis (AD) is a chronic inflammatory skin disorder that affects nearly 17% of children and can persist into adulthood. Advances in our understanding of mechanisms underlying AD require direct sampling of AD skin. Although AD is primarily a skin disease involving infants and young children, there are no skin-based studies examining AD in this age group because of the invasiveness of skin biopsies. The goal of this pilot study was to develop a mass spectrometry (MS)-based, noninvasive skin taping technique to study AD. Large-scale proteomic profiling can provide new hypotheses regarding AD pathogenesis and potentially identify new disease biomarkers.

Five patients with severe AD¹ (4 female, 1 male; median age, 45 years) and 5 normal nonatopic individuals with no history of atopic skin disease or allergies (3 female, 2 male; median age, 27 years) were recruited (Institutional Review Board protocol #HS 1962 NJ209). Four of 5 of the patients with AD had acute lesions (less than 3 days old), and all 5 had chronic (more than 3 days old) and nonlesional samples collected. All patients with AD had negative bacterial skin cultures and were required to discontinue the use of topical medications for 7 days and oral antibiotics for 10 days before sample collection.

A total of 20 standard D-Squame Skin Sampling Discs, 0.22-mm diameter (CuDerm, Dallas, Tex), were applied sequentially to each uncleaned chronic, acute, and nonlesional site of 5 patients with AD. Each disc was placed adhesive side up in its own borosilicate scintillation vial (Wheaton; Fisher Scientific, Pittsburgh, Pa) and frozen at -80°C . The same procedure was followed for the collection of tape discs from 3 nonatopic individuals at the same body locations sampled on the atopic patients (bicep, antecubital fossae, and back) and 2 nonatopic individuals from the antecubital fossae, for a total of 11 samples.

Proteins were extracted by using 0.01% 3-(3-[1,1-bisalkyloxethyl]pyridin-1-yl)propane-1-sulfonate (Protein Discovery, Knoxville, Tenn), a MS-compatible detergent. Extraction buffer was pooled from tape disc layers (1-5, 6-10, 11-15, and 16-20) to ensure sufficient material for MS analysis. Proteins were digested according to standard procedures.² MS analysis was performed in triplicate by using an HPLC-Chip ion trap instrument (Agilent Technologies, Santa Clara, Calif) coupled to an HPLC system (1200 series HPLC; Agilent). Protein identification was conducted by using the Spectrum Mill database search program (Agilent) to match mass spectral data to sequences in the Swiss Prot protein database. Database results were compiled for the replicate MS runs, pooled layers, patients, and lesions. The data were processed to calculate fold change between groups by using a Java (Sun Microsystems, Santa Clara, Calif) application developed in house. Proteins were further validated by using Scaffold (Proteome Software, Portland, Ore). Protein levels were compared between lesion type groups (acute, chronic, nonlesional atopic, and nonatopic) by using permutation F tests ($\alpha = 0.05$). Pairwise comparisons were performed by using 2-sample permutation tests. See this article's complete Methods in the Online Repository at www.jacionline.org.

A similar number of total proteins was identified with high confidence using the in-house Java application and Scaffold (104 vs 101 proteins, respectively; see this article's Table E1 in the Online Repository at www.jacionline.org). Fifty-three unique proteins were identified in patients with AD (corresponding to 370 unique peptides) and 4 in nonatopic subjects (corresponding to 34 unique peptides), and 44 proteins were common between the 2 groups. The biological processes represented by the identified proteins are depicted in this article's Fig E1 in the Online Repository at www.jacionline.org (Scaffold). Notable categories include immune response, response to stimulus, multiorganism response, and biological adhesion.

Proteins that met the inclusion criteria (see the Online Repository, Protein Selection) for novel, increased, and decreased protein levels between patient groups/lesion types were subjected to statistical analyses (dermcidin precursor, serpin B3 [squamous cell carcinoma antigen 1], triosephosphate isomerase, epidermal fatty acid binding protein [e-fabp], α -enolase, caspase 14 precursor, keratin type 1 cytoskeletal 10, and keratin type 2 cytoskeletal 1; see this article's Table E2 in the Online Repository at www.jacionline.org). All proteins yielded permutation F tests with $P \leq .05$ for overall group differences, except dermcidin ($P = .15$; see this article's Table E3 in the Online Repository at www.jacionline.org; Fig 1).

Consistent with a previous report by Yamane et al,³ 2 proteins were uniquely identified in patients with AD: e-fabp and α -enolase (Table E2). Triosephosphate isomerase and serpin B3 were increased in patients with AD compared with nonatopic controls (Table E2). Four proteins were decreased in the acute lesions of patients with AD compared with nonatopic subjects: keratin type I cytoskeletal 10, keratin type II cytoskeletal 1, caspase 14 precursor, and dermcidin precursor (Table E2). The elevated levels of serpin B3, a previously identified biomarker for AD,⁴ and decreased levels of the sweat-derived antimicrobial peptide dermcidin⁵ are consistent with the literature and serve to validate this proteomics technique further.

As reported in a previous study,³ α -enolase was found exclusively in the skin of patients with AD. It is plausible that human exposure to α -enolase on the surface of pathogens leads to development of autoantigens in the human body. In support of this theory, α -enolase has been shown to be autoantigenic in patients with psoriasis,⁶ and such an autoimmune reaction may perpetuate the chronic inflammation of AD.

In accordance with a previous study,³ e-fabp was highly expressed in acute and chronic lesions of patients with AD, was minimally expressed in the nonlesional skin of patients with AD, and was absent in nonatopic individuals. Fatty acid binding proteins are abundant intracellular proteins that bind and transport otherwise insoluble long-chain fatty acids and appear to be essential for normal keratinocyte differentiation.⁷ It has been proposed that fatty acid binding proteins may serve as master regulators of inflammatory signaling pathways.⁸ In support of this, e-fabp has been shown to bind and stabilize leukotriene A₄ and may modulate the production or metabolism of bioactive eicosanoids, which have been found in the urine of patients with AD.^{9,10} Limited studies have been published on the effects of leukotriene antagonists/synthesis inhibitors on the treatment of AD.

In conclusion, this proof-of-principle pilot study has revealed new proteins possibly involved in AD pathogenesis. These

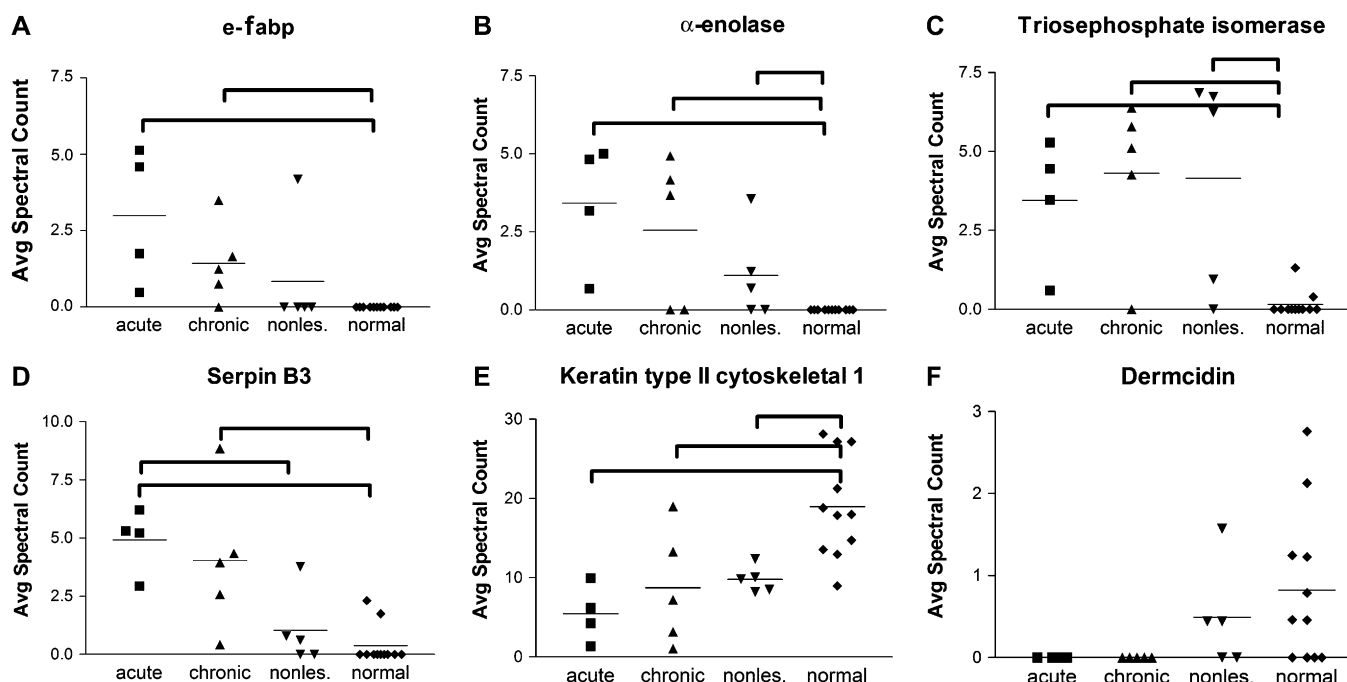


FIG 1. Quantification of proteins of interest. Figures represent average spectral count values for patients between groups. Significant differences between 2 groups using the Benjamini-Hochberg false discovery rate procedure with false discovery rate=0.05 within each protein indicated by bracket endpoints over those columns. See Methods for statistical analysis details. **A**, e-fabp. **B**, α -Enolase. **C**, Triosephosphate isomerase. **D**, Serpin B3. **E**, Keratin 1 type II cytoskeletal 1. **F**, Dermcidin. Avg, Average; nonles., nonlesional.

may serve as biomarkers for the development and persistence of AD. Studies are planned to assess a larger population of age-matched and sex-matched patient groups, especially children.

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METHODS

Sample collection and storage

Patients were directed to avoid showering the day of the collections. With the technicians wearing gloves and using ophthalmic curved forceps (World Precision Instruments, Inc, Sarasota, Fla), standard D-Squame Skin Sampling Discs, 0.22-mm diameter (CuDerm, Dallas, Tex), were applied to uncleaned chronic, acute (when present), and nonlesional sites of 5 patients with AD. A total of 20 separate tape discs were applied to each site. On application of the first tape disc, 4 marks were placed around the disc with pen so that subsequent discs could be applied to the same location. Each tape disc was placed adhesive side up in its own 20-mL borosilicate scintillation vial (Wheaton; Fisher Scientific, Pittsburgh, Pa) and frozen at -80°C . The same procedure was followed for the collection of tape discs from 3 nonatopic individuals at the same body locations sampled on the atopic subjects (bicep, antecubital fossae, and back) and 2 nonatopic individuals from the antecubital fossae for a total of 11 samples. For each patient, a set of 5 blank tape discs were used as a process control. D-Squame tape strips were chosen because they were developed to remove the stratum corneum. Their physical properties (size and rigidity) further enhanced their ease of use in the collection, storage, and extraction procedures. Furthermore, the adhesive on the discs did not interfere with the mass-spectrometric analysis.

Protein extraction

Proteins were extracted by using a buffer composed of 0.01% 3-(3-[1,1-bisalkyloxyethyl]pyridin-1-yl)propane-1-sulfonate (Protein Discovery, Knoxville, Tenn) in 50 mmol/L ammonium bicarbonate with 1x HALT protease inhibitors, EDTA-free (Pierce; Thermo Scientific, Rockford, Ill), and 50 mmol/L dithiothreitol (Bio-Rad, Hercules, Calif) and incubated on a rocker at room temperature for 1 hour. Extraction buffer was pooled from tape disc layers (1-5, 6-10, 11-15, and 16-20), transferred into polypropylene 1.7-mL microcentrifuge tubes, lyophilized, and stored at -80°C . Samples were pooled in groups of 5 to ensure sufficient material for mass-spectrometric analysis and to maximize the number of proteins that could be identified.

Protein digestion

Proteins were precipitated in 300 μL ice-cold precipitation buffer consisting of 0.1% formic acid in 80:20 methanol:water (VWR, West Chester, Pa; and Fisher Scientific, respectively). Samples were incubated at -20°C and vortexed for 30 seconds every 10 minutes for 1 hour, then centrifuged at 18,000g at 4°C for 20 minutes. The supernatant was removed, and the protein pellet was resuspended in 8 mol/L urea (Sigma Ultra, St Louis, Mo) in 100 mmol/L TRIS HCl (pH 8.5; Fisher Scientific). Proteins were reduced with 5 mmol/L TCEP (tris(2-carboxyethyl)phosphine) (Pierce) for 20 minutes and alkylated with 500 mmol/L iodoacetamide (Bio-Rad) for 15 minutes. Urea was diluted to 2 mol/L with 100 mmol/L TRIS HCl (pH 8.5), and samples were incubated at 37°C overnight with 100 ng trypsin (Trypsin Gold; Promega, Madison, Wis). Samples were then cleaned by using PepClean C-18 spin columns (Pierce) following the manufacturer's protocol. All reagents were of the highest grade available for MS.

MS

Samples were resuspended in 10 μL 0.1% formic acid (EMD, Gibbstown, NJ) in 3% acetonitrile (Fisher Scientific) and transferred into sample vials. Liquid chromatography was carried out using an Agilent 1200 series HPLC equipped with a capillary pump (pump A) and a nano pump (pump B; Agilent, Santa Clara, Calif). Eluted peptides were analyzed on an Agilent ion trap (model 6340) mass spectrometer with an HPLC-chip interface. A long HPLC chip was used with a 40-nL enrichment column and a 75- $\mu\text{m} \times 150\text{-mm}$ analytical column combined on a single chip. Buffer A for the nano pump consisted of 0.1% formic acid in HPLC grade water; buffer B contained 90% acetonitrile, 10% HPLC grade water, and 0.1% formic acid. The buffer for the capillary pump contained 3% acetonitrile, 97% HPLC grade water, and 0.1% formic acid. Ion trap parameters were as follows: voltage, 1750; drying gas,

5 L/min; temperature, 350°C ; m/z (mass to charge ratio) range (ms), 300 to 1600; m/z range (ms/ms), 50 to 2200; precursor ions, 4; active exclusion, yes; and scan rate, ultra (26,000 m/z per second). Gradient conditions for pump B were as follows: 3% B from 0 to 32 minutes, 40% B from 32.1 to 36 minutes, 80% B from 36.1 to 43 minutes, 80% B from 43.1 to 47 minutes, and 3% B from 47.1 to 50 minutes. A total of 1 μL of the prepared peptides was injected onto the enrichment column for concentration/purification. The chip cube was switched to redirect the flow from pump B to elute the enrichment column onto the analytical column from 0 to 50 minutes. Flow rates were 0.45 $\mu\text{L}/\text{min}$ for the nano pump and 4 $\mu\text{L}/\text{min}$ for the capillary pump. All samples were run in triplicate.

Database searching

Raw data were extracted and analyzed by using the Spectrum Mill database searching program (Rev A.03.03.080 SR1; Agilent, Santa Clara, Calif). Peak picking was performed within Spectrum Mill with the following parameters: signal-to-noise was set at 15:1, a maximum charge state of 4 was allowed ($z < 4$), and the program was directed to attempt to find a precursor charge state. During searching, the following parameters were applied: Swiss Prot Homo Sapiens database (UniProt Release 14), carbamidomethylation as a fixed modification, oxidized methionine as a variable modification, trypsin, maximum of 2 missed cleavages, precursor mass tolerance ± 2.5 , product mass tolerance ± 0.7 , and maximum ambiguous precursor charge of 3.

Protein selection

Database results were compiled for the replicate MS runs, pooled layers, patients, and lesions. The data were extensively processed to calculate fold change between groups. The data analysis steps are as follows: (1) Spectrum Mill results were exported into Excel, saved as tab-delimited text files, and imported *en masse* into an in-house Java application (Sun Microsystems), and (2) spectral counting was performed to quantitate relative protein amounts whereby the sum of the spectra matched to peptides representing a single protein in the database correlated to the amount of that protein present. After spectral counting data were normalized based on the total number of spectra matched to a protein divided by the total number of spectra per MS run (no. of protein spectra/total no. of MS spectra), (3) proteins were filtered to include only those present in 2 out of 3 technical replicates and with at least 2 peptides, (4) data from the 3 technical replicates were averaged, yielding SD and % coefficient of variation, and (5) lesion types (acute, chronic, nonlesional atopic, or normal/nonatopic) were averaged across patients, yielding SD and % coefficient of variation. Only non-0 values were averaged for the different lesions to avoid skewing the data. This is accounted for by including the number of patients each protein was found in along with the fold change for each protein between groups and layers. Then, (6) fold change was calculated by dividing the mean of all patients for that group by the mean value for all normal individuals.

Differentially expressed proteins were selected from the complete list of identified proteins. Selection criteria for differential protein expression were based on a minimum of 2-fold change up or down, present in 2 or more layers, and found in 75% or more patients for at least 1 lesional site. These criteria were developed to limit focus to proteins showing strong, reproducible changes across patients and layers. Scaffold (version Scaffold 2.04; Proteome Software Inc, Portland, Ore) was used to validate MS/MS-based peptide and protein identifications. Peptide identifications were accepted if they could be established at greater than 95.0% probability as specified by the Peptide Prophet algorithm.^{E1} Protein identifications were accepted if they could be established at greater than 99.9% probability and contained at least 2 identified peptides.

Statistical methods

Protein levels were compared between lesion type groups (acute, chronic, nonlesional atopic, and normal/nonatopic) by using permutation F tests.^{E2} This test was chosen instead of ANOVA because of the strong nonnormality of the data (0 values mixed with positive continuous values). Replicates

within subjects were averaged before analysis. Tests were conducted separately for each protein. First, an overall permutation F test was conducted to compare groups. If the test was significant at the $\alpha=0.05$ level, pairwise comparisons were then carried out by using 2-sample permutation tests, with the Benjamini-Hochberg procedure to control the false discovery rate (FDR). The more conservative Benjamini-Liu FDR procedure was also applied to verify results. The FDR was set to 0.05 for the set of tests within each protein. The Fisher exact generalized test was used to compare rates of 0 versus positive-valued responses between groups for those proteins that did have some 0 values (all except caspase 14, keratin 10, and keratin 1). Pairwise tests were conducted by using the Fisher exact 2×2 test for

proteins in which overall group differences were found, again controlling the FDR as described.

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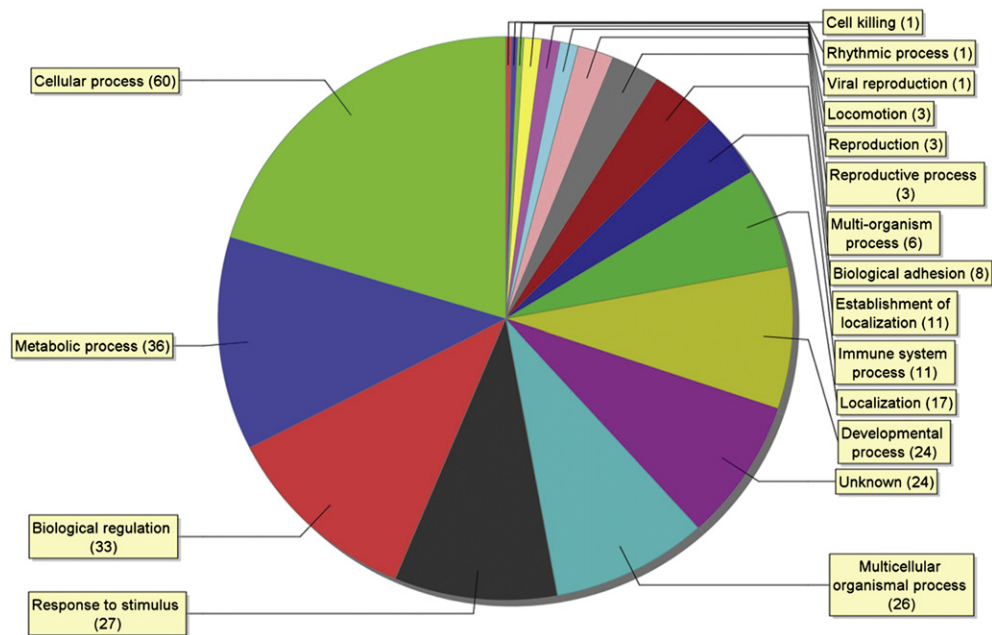


FIG E1. Biological processes of identified proteins. Notable processes include metabolic, immune response, response to stimulus, multiorganism response, and biological adhesion.

TABLE E1. Complete list of proteins identified among all samples

Accession no.	Protein name		Layers 1-5				Layers 6-10				Layers 11-15				Layers 16-20			
			Acute	Chronic	Nonles	N	Acute	Chronic	Nonles	N	Acute	Chronic	Nonles	N	Acute	Chronic	Nonles	N
P02768	Serum albumin precursor	Fold Δ	3.6	1.7	1.6	1.0	1.7	1.7	1.9	1.0	2.1	1.9	1.5	1.0	1.2	0.7	0.9	1.0
		%	75.0	60.0	80.0	54.5	75.0	80.0	80.0	27.3	50.0	100.0	60.0	54.5	75.0	100.0	100.0	63.6
P13645	Keratin, type I cytoskeletal 10	Fold Δ	0.5	0.6	0.5	1.0	0.4	0.6	0.5	1.0	0.4	0.6	0.6	1.0	0.5	0.6	0.6	1.0
		%	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	90.9	100.0	100.0	100.0	100.0
Q02413	Desmoglein-1 precursor	Fold Δ	1.1	0.8	0.9	1.0	1.7	1.4	1.6	1.0	1.4	1.1	1.2	1.0	1.6	1.5	1.5	1.0
		%	100.0	100.0	100.0	100.0	100.0	80.0	100.0	72.7	100.0	75.0	100.0	45.5	100.0	100.0	100.0	63.6
Q01469	e-fabp	Fold Δ	421.9	344.2	561.8	1.0	314.6	199.4	567.3	1.0	374.3	303.6	225.4	1.0	378.6	228.2	319.9	1.0
		%	75.0	60.0	20.0	0.0	100.0	60.0	20.0	0.0	75.0	25.0	20.0	0.0	75.0	75.0	20.0	0.0
P04264	Keratin, type II cytoskeletal 1	Fold Δ	0.3	0.6	0.5	1.0	0.3	0.6	0.5	1.0	0.3	0.4	0.4	1.0	0.5	0.5	0.6	1.0
		%	75.0	100.0	100.0	90.9	100.0	80.0	100.0	100.0	75.0	75.0	100.0	100.0	75.0	75.0	100.0	100.0
Q08554	Desmocollin-1 precursor	Fold Δ	1.1	0.9	1.0	1.0	1.2	0.9	1.3	1.0	1.5	1.0	1.5	1.0	1.6	1.3	1.4	1.0
		%	100.0	100.0	100.0	90.9	100.0	100.0	100.0	81.8	100.0	75.0	100.0	63.6	100.0	100.0	100.0	72.7
P25311	Zinc-α-2-glycoprotein precursor	Fold Δ	0.6	0.6	0.8	1.0	0.6	0.6	0.9	1.0	0.7	0.7	1.0	1.0	0.6	0.8	0.9	1.0
		%	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	75.0	100.0	90.9	100.0	100.0	100.0	100.0
P00738	Haptoglobin precursor	Fold Δ	426.291	1.000	1.000	1.0	1.000	1.000	1.000	1.0	1.000	203.273	1.000	1.0	0.007	0.007	0.007	1.0
		%	25.000	0.000	0.000	0.0	0.000	0.000	0.000	0.0	0.000	25.000	0.000	0.0	0.000	0.000	0.000	9.1
P31151	Protein S100-A7	Fold Δ	1.8	1.7	2.6	1.0	1.6	1.0	2.5	1.0	1.7	0.6	4.1	1.0	1.9	1.0	2.1	1.0
		%	50.0	60.0	40.0	18.2	50.0	20.0	40.0	27.3	25.0	25.0	40.0	18.2	50.0	75.0	40.0	18.2
P29508	Serpin B3	Fold Δ	5.5	8.1	4.0	1.0	1.6	1.6	0.3	1.0	2.5	2.2	0.004	1.0	2.0	2.3	1.8	1.0
		%	100.0	80.0	60.0	9.1	100.0	80.0	40.0	18.2	100.0	50.0	0.0	18.2	100.0	75.0	40.0	18.2
Q9NZT1	Calmodulin-like protein 5	Fold Δ	175.2	289.4	181.1	1.0	198.4	179.3	261.1	1.0	276.9	116.1	354.6	1.0	123.7	198.1	273.0	1.0
		%	50.0	20.0	20.0	0.0	75.0	20.0	20.0	0.0	25.0	25.0	20.0	0.0	50.0	25.0	20.0	0.0
P07339	Cathepsin D precursor	Fold Δ	1.4	1.1	0.9	1.0	1.3	1.1	1.0	1.0	1.2	0.9	0.8	1.0	0.9	0.9	0.9	1.0
		%	100.0	100.0	100.0	54.5	100.0	100.0	60.0	54.5	100.0	75.0	60.0	36.4	100.0	100.0	60.0	45.5
P01857	Ig γ-1 chain C region	Fold Δ	218.1	175.6	86.7	1.0	277.9	169.4	222.8	1.0	282.9	183.0	107.8	1.0	0.8	0.5	0.4	1.0
		%	75.0	60.0	20.0	0.0	50.0	80.0	20.0	0.0	50.0	75.0	40.0	0.0	75.0	75.0	40.0	9.1
P48666	Keratin, type II cytoskeletal 6C	Fold Δ	109.5	1.0	179.7	1.0	1.0	1.0	1.0	1.0	134.8	129.2	1.0	1.0	1.0	1.0	1.0	1.0
		%	25.0	0.0	20.0	0.0	0.0	0.0	0.0	0.0	25.0	25.0	0.0	0.0	0.0	0.0	0.0	0.0
Q15517	Corneodesmosin precursor	Fold Δ	0.5	0.3	0.6	1.0	0.7	0.6	0.9	1.0	1.0	0.5	1.0	1.0	0.8	0.5	0.7	1.0
		%	50.0	60.0	40.0	81.8	100.0	60.0	80.0	36.4	50.0	25.0	60.0	27.3	50.0	50.0	80.0	54.5
P31944	Caspase-14 precursor	Fold Δ	0.5	0.4	0.8	1.0	0.4	0.5	0.7	1.0	0.4	0.6	0.7	1.0	0.4	0.7	0.5	1.0
		%	100.0	100.0	100.0	100.0	100.0	100.0	80.0	100.0	75.0	75.0	100.0	90.9	75.0	100.0	100.0	81.8
Q9NZH8	IL-1 family member 9	Fold Δ	117.7	130.0	248.4	1.0	160.4	1.0	348.9	1.0	107.7	1.0	348.9	1.0	1.0	1.0	174.5	1.0
		%	25.0	20.0	20.0	0.0	25.0	0.0	20.0	0.0	50.0	0.0	20.0	0.0	0.0	0.0	20.0	0.0
P01834	Ig κ chain C region	Fold Δ	163.9	81.1	1.0	1.0	209.5	116.6	58.1	1.0	148.9	208.3	1.0	1.0	1.9	0.8	0.7	1.0
		%	75.0	40.0	0.0	0.0	50.0	40.0	20.0	0.0	50.0	50.0	0.0	0.0	50.0	25.0	20.0	9.1
P06702	Protein S100-A9	Fold Δ	1.5	2.1	4.0	1.0	0.5	0.01	0.01	1.0	1.0	1.0	1.0	1.0	117.4	1.0	121.1	1.0
		%	25.0	20.0	20.0	9.1	25.0	0.0	0.0	9.1	0.0	0.0	0.0	0.0	25.0	0.0	20.0	0.0
Q15828	Cystatin-M precursor	Fold Δ	0.9	0.8	0.7	1.0	0.9	0.8	0.9	1.0	1.2	1.2	1.3	1.0	1.1	1.2	1.2	1.0
		%	50.0	60.0	80.0	18.2	100.0	80.0	80.0	18.2	75.0	50.0	80.0	18.2	75.0	100.0	80.0	27.3
P07355	Annexin A2	Fold Δ	0.8	1.0	1.3	1.0	1.0	1.1	1.3	1.0	1.1	0.9	1.4	1.0	1.1	1.3	1.2	1.0
		%	100.0	100.0	80.0	63.6	100.0	80.0	80.0	72.7	100.0	75.0	80.0	54.5	100.0	100.0	100.0	63.6
Q06830	Peroxiredoxin-1	Fold Δ	163.6	118.1	1.0	1.0	228.9	69.9	1.0	1.0	174.5	102.4	150.3	1.0	1.0	115.2	100.3	1.0
		%	100.0	40.0	0.0	0.0	50.0	20.0	0.0	0.0	25.0	25.0	20.0	0.0	0.0	75.0	20.0	0.0

(Continued)

TABLE E1. (Continued)

Accession no.	Protein name		Layers 1-5				Layers 6-10				Layers 11-15				Layers 16-20			
			Acute	Chronic	Nonles	N	Acute	Chronic	Nonles	N	Acute	Chronic	Nonles	N	Acute	Chronic	Nonles	N
P00441	Superoxide dismutase [Cu-Zn]	Fold Δ	0.572	0.557	0.564	1.0	0.295	0.006	0.006	1.0	0.593	0.007	0.007	1.0	0.005	0.005	0.005	1.0
		%	50.000	20.000	20.000	9.1	25.000	0.000	0.000	27.3	25.000	0.000	0.000	27.3	0.000	0.000	0.000	36.4
P01876	Ig α-1 chain C region	Fold Δ	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	171.6	1.0	1.0	183.8	1.0	1.0	1.0
		%	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	25.0	0.0	0.0	25.0	0.0	0.0	0.0
P10599	Thioredoxin	Fold Δ	0.7	1.0	1.1	1.0	0.6	0.9	1.1	1.0	0.8	1.2	1.3	1.0	0.7	0.7	0.9	1.0
		%	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	50.0	100.0	81.8	100.0	100.0	100.0	90.9
P35908	Keratin, type II cytoskeletal 2 epidermal	Fold Δ	0.4	1.3	0.001	1.0	0.7	2.4	1.0	1.0	0.6	1.0	0.0	1.0	1.1	1.2	1.0	1.0
		%	50.0	20.0	0.0	18.2	25.0	20.0	40.0	9.1	25.0	25.0	0.0	9.1	50.0	50.0	40.0	27.3
P32119	Peroxiredoxin-2	Fold Δ	1.0	157.2	129.6	1.0	76.0	122.5	182.7	1.0	1.0	165.4	148.8	1.0	2.4	0.9	1.2	1.0
		%	0.0	60.0	60.0	0.0	25.0	60.0	40.0	0.0	0.0	50.0	60.0	0.0	25.0	25.0	40.0	9.1
P06733	α-Enolase	Fold Δ	403.7	420.5	234.8	1.0	372.4	411.7	202.0	1.0	327.0	468.7	263.9	1.0	366.3	436.7	194.8	1.0
		%	75.0	60.0	40.0	0.0	100.0	60.0	60.0	0.0	100.0	50.0	40.0	0.0	100.0	75.0	60.0	0.0
P63261	Actin, cytoplasmic 2	Fold Δ	128.2	1.0	1.0	1.0	129.0	180.9	103.9	1.0	218.9	171.8	116.3	1.0	217.3	123.6	1.0	1.0
		%	50.0	0.0	0.0	0.0	50.0	40.0	20.0	0.0	25.0	25.0	20.0	0.0	25.0	50.0	0.0	0.0
P04792	Heat shock protein β-1	Fold Δ	1.0	72.3	1.0	1.0	1.0	96.7	1.0	1.0	1.0	100.3	1.0	1.0	1.0	1.0	1.0	1.0
		%	0.0	20.0	0.0	0.0	0.0	20.0	0.0	0.0	0.0	25.0	0.0	0.0	0.0	0.0	0.0	0.0
P60174	Triosephosphate isomerase	Fold Δ	476.1	544.2	780.4	1.0	1.9	2.4	2.2	1.0	2.3	3.2	3.2	1.0	1.1	1.8	1.5	1.0
		%	75.0	80.0	60.0	0.0	75.0	80.0	80.0	9.1	100.0	75.0	80.0	9.1	100.0	100.0	80.0	9.1
O75223	Uncharacterized protein C7orf24	Fold Δ	1.0	0.9	1.2	1.0	1.2	1.0	1.1	1.0	1.0	0.8	1.1	1.0	1.1	1.1	1.0	1.0
		%	75.0	80.0	80.0	72.7	75.0	80.0	80.0	90.9	75.0	75.0	80.0	72.7	75.0	100.0	80.0	81.8
P05089	Arginase-1	Fold Δ	0.6	0.6	0.6	1.0	1.3	1.1	0.9	1.0	1.1	1.3	1.1	1.0	0.8	1.1	0.7	1.0
		%	75.0	80.0	80.0	18.2	75.0	60.0	80.0	54.5	75.0	50.0	80.0	72.7	75.0	75.0	80.0	54.5
Q5D862	Filaggrin-2	Fold Δ	0.5	0.6	0.8	1.0	0.4	0.4	1.0	1.0	0.5	0.4	0.9	1.0	0.6	0.6	0.7	1.0
		%	50.0	40.0	80.0	81.8	75.0	60.0	80.0	72.7	75.0	75.0	80.0	81.8	50.0	75.0	80.0	90.9
P19971	Thymidine phosphorylase	Fold Δ	183.1	141.3	126.0	1.0	245.7	195.0	88.5	1.0	247.2	1.0	1.0	1.0	202.0	151.5	62.8	1.0
		%	25.000	20.000	20.000	0.0	25.000	20.000	20.000	0.0	25.000	0.000	0.000	0.0	25.000	25.000	20.000	0.0
P02538	Keratin, type II cytoskeletal 6A	Fold Δ	91.7	1.0	80.7	1.0	0.001	0.122	0.001	1.0	1.0	1.0	1.0	1.0	0.003	0.003	0.003	1.0
		%	25.0	0.0	20.0	0.0	0.0	20.0	0.0	18.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	9.1
P50452	Serpin B8	Fold Δ	117.4	169.5	222.5	1.0	122.1	191.9	169.3	1.0	144.6	234.1	195.2	1.0	1.0	107.7	144.3	1.0
		%	25.0	40.0	40.0	0.0	25.0	40.0	40.0	0.0	25.0	25.0	40.0	0.0	0.0	50.0	40.0	0.0
Q08188	Protein-glutamine γ-glutamyl-transferase E	Fold Δ	0.5	0.5	0.3	1.0	0.5	0.4	0.3	1.0	0.6	0.4	1.1	1.0	0.5	0.6	0.4	1.0
		%	75.0	60.0	40.0	27.3	75.0	40.0	20.0	36.4	75.0	25.0	40.0	45.5	75.0	25.0	40.0	36.4
Q96P63	Serpin B12	Fold Δ	0.9	1.1	1.6	1.0	0.6	0.9	0.9	1.0	0.7	1.4	1.3	1.0	0.9	0.9	1.1	1.0
		%	50.0	40.0	60.0	54.5	50.0	40.0	80.0	63.6	50.0	25.0	60.0	81.8	25.0	50.0	80.0	72.7
Q9UI42	Carboxypeptidase A4	Fold Δ	78.4	80.9	1.0	1.0	1.0	102.1	72.7	1.0	59.5	1.0	63.2	1.0	1.0	151.5	1.0	1.0
		%	25.0	80.0	0.0	0.0	0.0	40.0	20.0	0.0	25.0	0.0	20.0	0.0	0.0	25.0	0.0	0.0
P56537	Eukaryotic translation initiation factor 6	Fold Δ	0.004	0.004	0.004	1.0	1.0	54.7	1.0	1.0	1.0	71.0	1.0	1.0	1.0	1.0	1.0	1.0
		%	0.0	0.0	0.0	9.1	0.0	20.0	0.0	0.0	0.0	25.0	0.0	0.0	0.0	0.0	0.0	0.0
P04406	Glyceraldehyde-3-phosphate dehydrogenase	Fold Δ	1.2	1.0	0.4	1.0	1.8	1.4	1.4	1.0	1.3	1.1	0.8	1.0	1.0	0.8	0.7	1.0
		%	75.0	80.0	60.0	9.1	75.0	80.0	60.0	36.4	75.0	75.0	80.0	36.4	75.0	100.0	80.0	27.3
P15924	Desmoplakin	Fold Δ	80.3	74.6	84.6	1.0	128.2	107.0	118.8	1.0	139.8	68.9	96.4	1.0	0.6	0.5	0.8	1.0
		%	50.0	20.0	40.0	0.0	25.0	40.0	20.0	0.0	25.0	25.0	20.0	0.0	25.0	50.0	40.0	18.2
P17900	Ganglioside GM2 activator	Fold Δ	51.9	1.0	1.0	1.0	1.0	1.0	1.0	1.0	58.2	1.0	1.0	1.0	1.0	1.0	1.0	1.0

(Continued)

TABLE E1. (Continued)

Accession no.	Protein name		Layers 1-5				Layers 6-10				Layers 11-15				Layers 16-20			
			Acute	Chronic	Nonles	N	Acute	Chronic	Nonles	N	Acute	Chronic	Nonles	N	Acute	Chronic	Nonles	N
P06396	Gelsolin	%	25.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	25.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
		Fold Δ	52.5	91.7	166.0	1.0	1.0	135.2	103.0	1.0	108.0	100.3	1.0	1.0	1.0	144.0	1.0	1.0
		%	25.0	60.0	20.0	0.0	0.0	40.0	20.0	0.0	25.0	25.0	0.0	0.0	0.0	25.0	0.0	0.0
Q6E0U4	Dermokine	Fold Δ	70.7	82.1	1.0	1.0	110.4	89.0	73.9	1.0	67.7	116.2	109.1	1.0	112.4	120.1	81.2	1.0
		%	25.0	40.0	0.0	0.0	25.0	60.0	40.0	0.0	50.0	50.0	40.0	0.0	25.0	75.0	40.0	0.0
		Fold Δ	0.439	0.005	0.708	1.0	0.005	0.005	0.279	1.0	0.006	0.006	0.006	1.0	0.006	0.006	0.006	1.0
P35527	Keratin, type I cytoskeletal 9	%	25.000	0.000	40.000	54.5	0.000	0.000	20.000	27.3	0.000	0.000	0.000	18.2	0.000	0.000	0.000	18.2
		Fold Δ	0.196	0.003	0.003	1.0	0.249	0.002	0.333	1.0	0.265	0.002	0.002	1.0	0.003	0.003	0.003	1.0
		%	25.000	0.000	0.000	45.5	25.000	0.000	20.000	18.2	25.000	0.000	0.000	9.1	0.000	0.000	0.000	18.2
P13647	Keratin, type II cytoskeletal 5	Fold Δ	0.004	0.5	1.0	1.0	0.004	0.86	0.9	1.0	0.01	1.3	0.8	1.0	0.005	1.1	1.4	1.0
		%	0.0	40.0	20.0	36.4	0.0	20.0	40.0	18.2	0.0	25.0	20.0	36.4	0.0	25.0	20.0	36.4
		Fold Δ	1.053	0.599	0.729	1.0	2.180	0.793	0.010	1.0	168.886	174.801	64.260	1.0	117.431	86.430	1.000	1.0
Q14574	Desmocollin-3	%	50.000	60.000	60.000	9.1	50.000	40.000	0.000	9.1	50.000	25.000	20.000	0.0	25.000	50.000	0.000	0.0
		Fold Δ	0.012	0.913	0.012	1.0	1.000	63.920	59.553	1.0	1.000	1.000	1.000	1.0	1.000	1.000	1.000	1.0
		%	0.0	40.0	0.0	9.1	0.0	40.0	20.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
O95274	Ly6/PLAUR domain-containing protein 3	Fold Δ	0.01	0.7	0.01	1.0	0.01	0.01	0.01	1.0	0.4	0.01	0.01	1.0	0.01	0.01	0.01	1.0
		%	0.0	20.0	0.0	9.1	0.0	0.0	0.0	18.2	25.0	0.0	0.0	9.1	0.0	0.0	0.0	18.2
		Fold Δ	0.909	1.188	1.446	1.0	0.769	0.857	0.997	1.0	0.646	0.831	0.916	1.0	0.672	1.408	1.135	1.0
Q9H0W9	Ester hydrolase C11orf54	%	50.000	40.000	60.000	45.5	50.000	60.000	80.000	72.7	75.000	50.000	80.000	63.6	75.000	50.000	80.000	90.9
		Fold Δ	0.003	0.003	0.003	1.0	0.003	0.260	0.257	1.0	0.005	0.005	0.005	1.0	0.009	0.009	0.009	1.0
		%	0.000	0.000	0.000	18.2	0.000	20.000	20.000	18.2	0.000	0.000	0.000	27.3	0.000	0.000	0.000	9.1
P68871	Hemoglobin subunit β	Fold Δ	0.0	0.0	0.0	1.0	0.0	0.6	0.4	1.0	0.3	2.1	0.5	1.0	0.7	0.1	0.4	1.0
		%	0.0	0.0	0.0	9.1	0.0	40.0	20.0	9.1	25.0	50.0	20.0	18.2	25.0	25.0	40.0	18.2
		Fold Δ	1.0	173.3	1.0	1.0	55.2	167.2	1.0	1.0	1.0	135.1	1.0	1.0	187.6	306.3	1.0	1.0
P31947	14-3-3 protein sigma	%	0.0	20.0	0.0	0.0	25.0	40.0	0.0	0.0	0.0	50.0	0.0	0.0	25.0	25.0	0.0	0.0
		Fold Δ	186.2	159.4	1.0	1.0	293.5	138.0	1.0	1.0	331.9	1.0	1.0	1.0	179.2	1.0	1.0	1.0
		%	25.0	20.0	0.0	0.0	25.0	20.0	0.0	0.0	25.0	0.0	0.0	0.0	25.0	0.0	0.0	0.0
P68133	Actin, α skeletal muscle	Fold Δ	1.0	131.4	109.2	1.0	134.0	1.0	1.0	1.0	136.1	1.0	1.0	1.0	151.1	1.0	62.8	1.0
		%	0.0	20.0	20.0	0.0	25.0	0.0	0.0	0.0	25.0	0.0	0.0	0.0	25.0	0.0	20.0	0.0
		Fold Δ	1.0	86.8	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
P27482	Calmodulin-like protein 3	%	0.0	20.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
		Fold Δ	2.4	2.9	1.9	1.0	1.0	1.7	1.1	1.0	0.9	1.1	0.6	1.0	1.3	0.7	0.9	1.0
		%	25.0	20.0	60.0	9.1	25.0	20.0	40.0	45.5	25.0	25.0	60.0	54.5	25.0	50.0	80.0	63.6
P09211	Glutathione S-transferase P	Fold Δ	73.369	87.528	1.000	1.0	1.000	87.467	1.000	1.0	87.174	73.520	1.000	1.0	136.842	1.000	1.000	1.0
		%	25.0	20.0	0.0	0.0	0.0	40.0	0.0	0.0	25.0	25.0	0.0	0.0	50.0	0.0	0.0	0.0
		Fold Δ	1.0	1.0	1.0	1.0	0.01	0.01	1.02	1.00	0.01	0.31	0.79	1.00	0.01	1.0	0.9	1.0
P20930	Filaggrin	%	0.0	0.0	0.0	0.0	0.0	0.0	40.0	9.1	0.0	25.0	20.0	9.1	0.0	50.0	40.0	36.4
		Fold Δ	1.0	128.9	58.7	1.0	1.000	1.000	132.144	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
		%	0.0	20.0	20.0	0.0	0.0	0.0	20.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
P35237	Serpine B6	Fold Δ	0.01	1.14	0.01	1.0	390.5	1.0	87.1	1.0	101.5	1.0	1.0	1.0	156.9	1.0	247.1	1.0
		%	0.0	20.0	0.0	9.1	25.0	0.0	20.0	0.0	25.0	0.0	0.0	0.0	25.0	0.0	20.0	0.0
		Fold Δ	1.0	50.4	1.0	1.0	1.0	1.0	1.0	1.0	1.000	1.000	1.000	1.0	1.0	1.0	1.0	1.0
Q9GZP4	UPF0424 protein C1orf128	%	0.0	20.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
		Fold Δ	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	115.0	1.0	1.0	1.0	72.1	1.0	1.0
		%	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	25.0	0.0	0.0	0.0	25.0	0.0	0.0
Q6UWP8	Suprabasin	Fold Δ	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	115.0	1.0	1.0	1.0	72.1	1.0	1.0
		%	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	25.0	0.0	0.0	0.0	25.0	0.0	0.0

(Continued)

TABLE E1. (Continued)

Accession no.	Protein name		Layers 1-5				Layers 6-10				Layers 11-15				Layers 16-20			
			Acute	Chronic	Nonles	N	Acute	Chronic	Nonles	N	Acute	Chronic	Nonles	N	Acute	Chronic	Nonles	N
Q99497	Protein DJ-1	Fold Δ	1.0	1.0	81.9	1.0	0.01	0.01	0.01	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
		%	0.0	0.0	40.0	0.0	0.0	0.0	0.0	9.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
P23490	Loricrin	Fold Δ	1.0	63.4	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
		%	0.0	20.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
P01860	Ig γ -3 chain C region	Fold Δ	1.000	1.000	1.000	1.0	1.000	1.000	1.000	1.0	0.008	0.008	0.008	1.0	1.000	1.000	1.000	1.0
		%	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	9.1	0.0	0.0	0.0	0.0
P47929	Galectin-7	Fold Δ	1.0	72.9	1.0	1.0	0.015	1.414	1.294	1.0	1.0	83.6	97.9	1.0	1.0	69.8	112.7	1.0
		%	0.0	20.0	0.0	0.0	0.0	20.0	20.0	9.1	0.0	25.0	40.0	0.0	0.0	50.0	60.0	0.0
P00558	Phosphoglycerate kinase 1	Fold Δ	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	122.5	1.0	1.0	1.0	89.2	1.0	1.0	1.0
		%	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	25.0	0.0	0.0	0.0	25.0	0.0	0.0	0.0
P07384	Calpain-1 catalytic subunit	Fold Δ	1.000	1.000	62.745	1.0	1.000	1.000	1.000	1.0	1.000	1.000	1.000	1.0	1.000	1.000	1.000	1.0
		%	0.0	0.0	20.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
P81605	Dermcidin precursor	Fold Δ	0.003	0.003	0.720	1.0	0.005	0.005	0.005	1.0	0.008	0.008	2.486	1.0	0.007	0.007	0.007	1.0
		%	0.0	0.0	60.0	63.6	0.0	0.0	0.0	36.4	0.0	0.0	20.0	27.3	0.0	0.0	0.0	18.2
P05109	Protein S100-A8	Fold Δ	1.000	1.000	142.246	1.0	0.004	0.004	0.004	1.0	1.000	1.000	1.000	1.0	176.147	1.000	1.000	1.0
		%	0.0	0.0	20.0	0.0	0.0	0.0	0.0	9.1	0.0	0.0	0.0	0.0	25.0	0.0	0.0	0.0
Q8WWY7	WAP 4-disulfide core domain protein 12	Fold Δ	1.000	1.000	118.285	1.0	1.000	1.000	173.423	1.0	1.000	1.000	268.053	1.0	1.000	1.000	1.000	1.0
		%	0.0	0.0	20.0	0.0	0.0	0.0	20.0	0.0	0.0	0.0	20.0	0.0	0.0	0.0	0.0	0.0
O75608	Acyl-protein thioesterase 1	Fold Δ	1.0	1.0	103.7	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
		%	0.0	0.0	20.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
P61970	Nuclear transport factor 2	Fold Δ	1.0	1.0	133.7	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
		%	0.0	0.0	20.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
P35754	Glutaredoxin-1	Fold Δ	0.01	0.01	0.37	1.00	0.01	0.01	0.01	1.00	0.01	0.01	0.01	1.00	0.01	0.01	0.01	1.00
		%	0.0	0.0	20.0	9.1	0.0	0.0	0.0	9.1	0.0	0.0	0.0	9.1	0.0	0.0	0.0	9.1
P12273	Prolactin-inducible protein	Fold Δ	0.004	0.004	0.004	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
		%	0.0	0.0	0.0	27.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
A6NMY6	Putative annexin A2-like protein	Fold Δ	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	0.005	0.005	0.005	1.0	0.004	0.004	0.004	1.0
		%	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	9.1	0.0	0.0	0.0	9.1
P05090	Apolipoprotein D	Fold Δ	0.003	0.003	0.003	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
		%	0.0	0.0	0.0	9.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
P48594	Serpin B4	Fold Δ	1.0	1.0	1.0	1.0	1.0	1.0	261.1	1.0	1.0	153.8	67.7	1.0	1.0	1.0	1.0	1.0
		%	0.0	0.0	0.0	0.0	0.0	0.0	20.0	0.0	0.0	25.0	20.0	0.0	0.0	0.0	0.0	0.0
P69905	Hemoglobin subunit α	Fold Δ	0.005	0.005	0.005	1.0	0.002	1.0	0.5	1.0	0.4	1.7	0.4	1.0	0.4	0.1	0.3	1.0
		%	0.0	0.0	0.0	9.1	0.0	40.0	20.0	18.2	25.0	50.0	20.0	18.2	25.0	25.0	40.0	18.2
Q7Z794	Keratin, type II cytoskeletal 1b	Fold Δ	0.01	0.01	0.01	1.0	0.01	0.8	0.01	1.0	0.01	0.9	0.3	1.0	0.00	0.7	0.3	1.0
		%	0.0	0.0	0.0	18.2	0.0	20.0	0.0	36.4	0.0	25.0	20.0	45.5	0.0	25.0	20.0	45.5
P80188	Neutrophil gelatinase-associated lipocalin precursor	Fold Δ	1.0	1.0	1.0	1.0	320.6	1.0	1.0	1.0	388.1	1.0	1.0	1.0	215.6	1.0	321.6	1.0
		%	0.0	0.0	0.0	0.0	25.0	0.0	0.0	0.0	25.0	0.0	0.0	0.0	25.0	0.0	20.0	0.0
Q15782	Chitinase-3-like protein 2 precursor	Fold Δ	1.0	1.0	1.0	1.0	230.2	1.0	1.0	1.0	232.9	1.0	1.0	1.0	176.1	1.0	174.5	1.0
		%	0.0	0.0	0.0	0.0	25.0	0.0	0.0	0.0	25.0	0.0	0.0	0.0	25.0	0.0	20.0	0.0
P07858	Cathepsin B precursor	Fold Δ	1.0	1.0	1.0	1.0	160.200	1.000	1.000	1.0	107.964	1.000	1.000	1.0	1.0	1.0	223.0	1.0
		%	0.0	0.0	0.0	0.0	25.0	0.0	0.0	0.0	25.0	0.0	0.0	0.0	0.0	0.0	20.0	0.0

(Continued)

TABLE E1. (Continued)

Accession no.	Protein name		Layers 1-5				Layers 6-10				Layers 11-15				Layers 16-20			
			Acute	Chronic	Nonles	N	Acute	Chronic	Nonles	N	Acute	Chronic	Nonles	N	Acute	Chronic	Nonles	N
P13796	Plastin-2	Fold Δ	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
		%	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
P23381	Tryptophanyl-tRNA synthetase, cytoplasmic	Fold Δ	1.0	1.0	1.0	1.0	55.2	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
		%	0.0	0.0	0.0	0.0	25.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
P01009	α-1-Antitrypsin	Fold Δ	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	102.9	1.0	1.0	0.8	0.7	1.3	1.0
		%	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	25.0	0.0	0.0	25.0	25.0	20.0	9.1
P01842	Ig λ chain C regions	Fold Δ	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	153.8	1.0	1.0	260.3	54.1	1.0	1.0
		%	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	25.0	0.0	0.0	25.0	25.0	0.0	0.0
P02647	Apolipoprotein A-I	Fold Δ	1.000	1.000	1.000	1.0	1.000	1.000	88.511	1.0	1.000	1.000	85.405	1.0	1.000	1.000	96.597	1.0
		%	0.0	0.0	0.0	0.0	0.0	0.0	20.0	0.0	0.0	0.0	20.0	0.0	0.0	0.0	20.0	0.0
P01024	Complement C3	Fold Δ	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	101.8	1.0	1.0	1.0	1.0	1.0	1.0
		%	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	25.0	0.0	0.0	0.0	0.0	0.0	0.0
P04075	Fructose-bisphosphate aldolase A	Fold Δ	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	69.6	1.0	1.0	1.0	143.4	1.0	1.0	1.0
		%	0.000	0.000	0.000	0.0	0.000	0.000	0.000	0.0	25.000	0.000	0.000	0.0	25.000	0.000	0.000	0.0
P50395	Rab GDP dissociation inhibitor β	Fold Δ	1.000	1.000	1.000	1.0	1.000	1.000	1.000	1.0	69.630	1.000	1.000	1.0	1.000	1.000	1.000	1.0
		%	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	25.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
P00915	Carbonic anhydrase 1	Fold Δ	1.000	1.000	1.000	1.0	1.000	251.081	1.000	1.0	1.000	405.398	1.000	1.0	1.000	1.000	1.000	1.0
		%	0.0	0.0	0.0	0.0	0.0	20.0	0.0	0.0	0.0	25.0	0.0	0.0	0.0	0.0	0.0	0.0
P63104	14-3-3 Protein ζ/δ	Fold Δ	1.000	1.000	1.000	1.0	1.000	1.000	1.000	1.0	1.000	87.166	1.000	1.0	1.000	1.000	1.000	1.0
		%	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	25.0	0.0	0.0	0.0	0.0	0.0	0.0
Q92820	γ-Glutamyl hydrolase	Fold Δ	1.000	1.000	1.000	1.0	0.007	0.007	0.007	1.0	0.007	0.007	0.007	1.0	1.000	1.000	1.000	1.0
		%	0.0	0.0	0.0	0.0	0.0	0.0	0.0	9.1	0.0	0.0	0.0	9.1	0.0	0.0	0.0	0.0
Q14533	Keratin, type II cuticular Hb1	Fold Δ	1.000	1.000	1.000	1.0	1.000	1.000	1.000	1.0	1.000	1.000	241.144	1.0	1.000	1.000	1.000	1.0
		%	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	20.0	0.0	0.0	0.0	0.0	0.0
Q15323	Keratin, type I cuticular Ha1	Fold Δ	1.000	1.000	1.000	1.0	1.000	1.000	1.000	1.0	1.000	1.000	239.480	1.0	1.000	1.000	1.000	1.0
		%	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	20.0	0.0	0.0	0.0	0.0	0.0
P48163	NADP-dependent malic enzyme	Fold Δ	1.000	1.000	1.000	1.0	1.000	68.774	1.000	1.0	1.000	1.000	1.000	1.0	1.000	1.000	1.000	1.0
		%	0.0	0.0	0.0	0.0	0.0	20.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
P20933	N(4)-(β-N-acetylglucosaminy)-L-asparaginase	Fold Δ	1.000	1.000	1.000	1.0	1.000	1.000	1.000	1.0	0.010	0.010	0.010	1.0	1.000	1.000	1.000	1.0
		%	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	9.1	0.0	0.0	0.0	0.0
P02679	Fibrinogen γ chain	Fold Δ	1.000	1.000	1.000	1.0	1.000	1.000	1.000	1.0	1.000	1.000	1.000	1.0	61.540	1.000	1.000	1.0
		%	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	25.0	0.0	0.0	0.0
P62937	Peptidyl-prolyl cis-trans isomerase A	Fold Δ	1.000	1.000	1.000	1.0	1.000	55.905	1.000	1.0	1.000	1.000	1.000	1.0	1.000	1.000	1.000	1.0
		%	0.0	0.0	0.0	0.0	0.0	20.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
O75369	Filamin-B	Fold Δ	1.000	1.000	1.000	1.0	0.004	0.004	0.004	1.0	1.000	1.000	1.000	1.0	1.000	1.000	1.000	1.0
		%	0.0	0.0	0.0	0.0	0.0	0.0	0.0	9.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

%, Percentage of patients that the protein was found in; *Fold Δ*, fold change; *N*, normal, nonatopic; *Nonles*, nonlesional skin from atopic patient.

Criteria for inclusion were 2 or more peptides and present in 2 out of 3 technical replicates for a sample. Columns represent pooled layers of skin (ie, 1-5, 6-10, 11-15, and 16-20). Boxes highlighted *dark gray* indicate 2-fold or greater increase in protein expression; boxes highlighted *light gray* indicate 2-fold or greater decrease in protein expression. *GM2*, Ganglioside membrane 2; *Ly6/PLAUR*, Ly-6/urokinase-type plasminogen activator receptor; *Ly6/uPAR*, Ly-6/urokinase-type plasminogen activator receptor; *WAP*, whey acidic protein 2; *tRNA*, transfer ribonucleic acid; *Rab/GDP*, RAS oncogene in the brain/guanosine diphosphate; *Hb1*, hair basic 1; *Ha*, hair acidic; *NADP*, nicotinamide adenine dinucleotide phosphate.

TABLE E2. Differential protein expression in AD

Table 2. Differential protein expression in AD																		
			Layers 1-5				Layers 6-10				Layers 11-15				Layers 16-20			
Accession no.	Protein name		Acute	Chronic	Nonles	N	Acute	Chronic	Nonles	N	Acute	Chronic	Nonles	N	Acute	Chronic	Nonles	N
Up in AD																		
P29508	Serpin B3	Fold Δ	5.5	8.1	4.0	1.0	1.6	1.6	0.3	1.0	2.5	2.2	0.004	1.0	2.0	2.3	1.8	1.0
		%	100.0	80.0	60.0	9.1	100.0	80.0	40.0	18.2	100.0	50.0	0.0	18.2	100.0	75.0	40.0	18.2
P60174	Triosephosphate isomerase	Fold Δ	476.1	544.2	780.4	476.1	1.9	2.4	2.2	1.0	2.3	3.2	3.2	1.0	1.1	1.8	1.5	1.0
		%	75.0	80.0	60.0	0.0	75.0	80.0	80.0	9.1	100.0	75.0	80.0	9.1	100.0	100.0	80.0	9.1
Down in AD																		
P13645	Keratin, type I cytoskeletal 10	Fold Δ	0.5	0.6	0.5	1.0	0.4	0.6	0.5	1.0	0.4	0.6	0.6	1.0	0.5	0.6	0.6	1.0
		%	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	90.9	100.0	100.0	100.0	100.0
P04264	Keratin, type II cytoskeletal 1	Fold Δ	0.3	0.6	0.5	1.0	0.3	0.6	0.5	1.0	0.3	0.4	0.4	1.0	0.5	0.5	0.6	1.0
		%	75.0	100.0	100.0	90.9	100.0	80.0	100.0	100.0	75.0	75.0	100.0	100.0	75.0	75.0	100.0	100.0
P31944	Caspase 14 precursor	Fold Δ	0.5	0.4	0.8	1.0	0.4	0.5	0.7	1.0	0.4	0.6	0.7	1.0	0.4	0.7	0.5	1.0
		%	100.0	100.0	100.0	100.0	100.0	100.0	80.0	100.0	75.0	75.0	100.0	90.9	75.0	100.0	100.0	81.8
P81605	Dermcidin precursor	Fold Δ	0.003	0.003	0.720	1.0	0.005	0.005	0.005	1.0	0.008	0.008	2.486	1.0	0.007	0.007	0.007	1.0
		%	0.0	0.0	60.0	63.6	0.0	0.0	0.0	36.4	0.0	0.0	20.0	27.3	0.0	0.0	0.0	18.2
Unique in AD																		
Q01469	e-fabp	Fold Δ	421.9	344.2	561.8	1.0	314.6	199.4	567.3	1.0	374.3	303.6	225.4	1.0	378.6	228.2	319.9	1.0
		%	75.0	60.0	20.0	0.0	100.0	60.0	20.0	0.0	75.0	25.0	20.0	0.0	75.0	75.0	20.0	0.0
P06733	α-Enolase	Fold Δ	403.7	420.5	234.8	1.0	372.4	411.7	202.0	1.0	327.0	468.7	263.9	1.0	366.3	436.7	194.8	1.0
		%	75.0	60.0	40.0	0.0	100.0	60.0	60.0	0.0	100.0	50.0	40.0	0.0	100.0	75.0	60.0	0.0

%, Percentage of patients the protein was found in for that lesion type; *Fold Δ*, average normalized spectra for lesion type/average normalized spectra for all normals; *N*, normal, nonatopic; *Nonles*, nonlesional skin from atopic patient. Criteria for inclusion: 2 or more peptides, present in 2 out of 3 technical replicates, 2-fold change, present in 75% biological replicates in 2 or more pooled layers of skin. Boxes highlighted *dark gray* indicate 2-fold or greater increase in protein expression; boxes highlighted *light gray* indicate 2-fold or greater decrease in protein expression.

TABLE E3. *P* values from overall and pairwise tests to compare lesion type groups, using the permutation F test and Fisher exact test methods

Protein	Overall test	A vs C	A vs NL	A vs N	C vs NL	C vs N	NL vs N
Permutation F test [†]							
Dermcidin	0.15	NT	NT	NT	NT	NT	NT
Serpin B3	0.004	0.62	0.02*	0.001*	0.08	0.0008*	0.27
Trios.isom**	0.01	0.53	0.73	0.001*	0.97	0.0002*	0.003*
e-fabp	0.03	0.22	0.17	0.0004*	0.48	0.004*	0.31
α-Enolase	0.01	0.60	0.08	<0.0001*	0.31	0.02 [‡]	0.01 [‡]
Caspase 14	0.05	0.53	0.20	0.02	0.39	0.04	0.13
Keratin 10	0.04	0.46	0.12	0.02	0.83	0.07	0.03
Keratin 1	0.01	0.48	0.03	0.003*	0.77	0.01*	0.01*
Fisher exact test							
Dermcidin	0.03	NA [§]	0.17	0.07	0.17	0.03	1
Serpin B3	0.001	NA [§]	0.44	0.011*	0.44	0.005*	0.25
Trios.isom	0.01	1	1	0.01	1	0.04	0.04
e-fabp	0.00005	1	0.05	0.0007*	0.20	0.0027*	0.31
α-Enolase	0.0002	0.44	0.44	0.0007*	1	0.02 [‡]	0.02 [‡]

A, Acute; C, chronic; N, normal; NA, not applicable; NL, nonlesional; NT, not tested.

Also see "Statistical methods." $\alpha = 0.05$ for overall test.

*Significant with the Benjamini-Hochberg and Benjamini-Liu procedures using FDR = 0.05 for all pairwise comparisons within the protein.

[†]All tests used 5000 resamples for the permutation distribution.

[‡]Significant with the Benjamini-Hochberg procedure but not the Benjamini-Liu procedure using FDR = 0.05 for all pairwise comparisons within the protein.

[§]Responses were either all 0 or all greater than 0 for both groups.

**Triosephosphate isomerase.