Comparative proteomic profiling of patients with atopic dermatitis based on history of eczema herpeticum infection and *Staphylococcus aureus* colonization

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Background: Atopic dermatitis (AD) is the most common inflammatory skin disorder in the general population worldwide, and the majority of patients are colonized with *Staphylococcus aureus*. Eczema herpeticum is a disseminated herpes simplex virus infection that occurs in a small subset of patients.

Objectives: The goal was to conduct proteomic profiling of patients with AD based on *S aureus* colonization status and history of eczema herpeticum. We hoped to identify new biomarkers for improved diagnosis and prediction of eczema herpeticum and *S aureus* susceptibility and to generate new hypotheses regarding disease pathogenesis.

Methods: Skin taping was performed on nonlesional skin of nonatopic control subjects and on lesional and nonlesional skin of patients with AD. Subjects were classified according to the history of eczema herpeticum and *S aureus* colonization. Proteins were analyzed by using mass spectrometry; diagnostic groups were compared for statistically significant differences in protein expression.

Results: Proteins related to the skin barrier (filaggrin-2, corneodesmosin, desmoglein-1, desmocollin-1, and transglutaminase-3) and generation of natural moisturizing factor (arginase-1, caspase-14, and gamma-glutamyl cyclotransferase) were expressed at significantly lower levels in lesional versus nonlesional sites of patients with AD with and without history of eczema herpeticum; epidermal fatty acid–binding protein was expressed at significantly higher levels in patients with methicillin-resistant *S aureus*.

Conclusion: This noninvasive, semiquantitative profiling method has revealed novel proteins likely involved in the pathogenesis of AD. The lower expression of skin barrier proteins and enzymes involved in the generation of the natural moisturizing factor could further exacerbate barrier defects and perpetuate water loss from the skin. The greater expression of epidermal fatty acid–binding protein, especially in patients colonized with methicillin-resistant *S aureus*, might perpetuate the inflammatory response through eicosanoid signaling.

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Key words: *Atopic dermatitis, mass spectrometry, proteomics, natural moisturizing factor, eczema herpeticum, tape stripping, skin barrier, filaggrin-2, epidermal fatty acid binding protein, methicillin-resistant* Staphylococcus aureus

Atopic dermatitis (AD) is a chronic inflammatory skin disorder that affects nearly 17% of children and can persist into adulthood,[1] significantly compromising quality of life.[2] AD is a multifactorial skin disease characterized by defects in the skin barrier and immune system.[3] Numerous factors modulate disease severity on an individual basis, including genetic susceptibility,[4] immune response,[5] and diverse environmental factors.[3] Patients with AD are prone to skin infections, including eczema herpeticum (EH), a disseminated herpes simplex virus 1 or 2 infection that occurs in a subset of patients with AD.[6] EH can be complicated by keratoconjunctivitis, viremia, meningitis, and encephalitis.[7] Patients with EH tend to have early-onset AD, more severe disease, increased risk of asthma, increased allergen sensitization, increased T_H2 polarity, and more frequent skin infections.[8] Additionally, it has been shown that up to 90% of patients with AD are colonized with *S aureus*[9] and 16% are colonized with methicillin-resistant *Staphylococcus aureus* (MRSA).[10] Patients with a history of EH have a higher risk of MRSA colonization.[8]

In addition to an increased susceptibility to skin infection, patients with AD have numerous abnormalities in their epidermis, which acts as a critical mechanical barrier against microbes and serves to maintain proper skin hydration.[11] The epidermis is comprised of 4 distinct layers: basal (the deepest layer), spinous, granular, and cornified (the uppermost layer). Epidermal differentiation begins with the migration of proliferating keratinocytes from the basal layer and ends with their terminal differentiation into corneocytes (dead keratinocytes). The stratum corneum, or cornified layer, is a flattened sheet of corneocytes tightly connected by corneodesmosomes and embedded in an intercellular matrix of nonpolar lipids.[12] This layer of dead cells is the key physical and permeability barrier against the environment and is continuously shed and renewed by differentiating keratinocytes. Recent work suggests that abnormal epidermal differentiation, including defective corneocyte compaction, cornification, and lipid release, play a key role in the pathogenesis of AD.[13]

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Abbreviations used
AD: Atopic dermatitis
EASI: Eczema Area and Severity Index
e-fabp: Epidermal fatty acid binding protein
EH: Eczema herpeticum
EH−: AD without a history of eczema herpeticum
EH+: AD with a history of eczema herpeticum
flg-2: Filaggrin-2
GGCT: Gamma-glutamyl cyclotransferase
MRSA: Methicillin-resistant *Staphylococcus aureus*
MSSA: Methicillin-sensitive *Staphylococcus aureus*
NMF: Natural moisturizing factor
PCA: 2-Pyrrolidone-5-carboxylic acid
TG3: Transglutaminase-3 (protein-glutamine gamma-glutamyltransferase E)

The goal of this exploratory, hypothesis-generating proteomics study funded by the National Institutes of Health/National Institute of Allergy and Infectious Diseases' Atopic Dermatitis Vaccinia Network was to identify unique patterns of biomarkers associated with AD pathogenesis and EH/*S aureus* susceptibility. Samples were collected from nonatopic subjects and patients with AD by means of tape stripping, and proteomic profiling was performed. Samples were analyzed in triplicate by means of mass spectrometry, and a custom-designed, in-house Java Application was developed to process the data.[14] Differences in protein expression between diagnostic groups were estimated, and statistical significance was evaluated based on a linear mixed model.

METHODS

Study population and design

Participants with AD and nonatopic healthy control subjects aged 1 to 80 years were enrolled at National Jewish Health. AD was diagnosed according to standardized criteria developed by the National Institutes of Health/National Institute of Allergy and Infectious Diseases' Atopic Dermatitis and Vaccinia Network.[8] A total of 65 participants were enrolled: 29 patients with AD without a history of eczema herpeticum (EH− patients), 21 patients with AD with a history of eczema herpeticum (EH+ patients), and 15 nonatopic control subjects. Swabs were collected from nonlesional skin of all participants and lesional skin of participants with AD to determine *S aureus* colonization status (methicillin-sensitive *Staphylococcus aureus* [MSSA]/MRSA/no *S aureus* colonization). Skin tapings were collected from nonlesional and lesional skin (if applicable). Participants were required to discontinue the use of topical medications for 7 days and oral antibiotics for 10 days before sample collection.

Proteomic analysis was conducted on skin tapings from a subset of participants who were sex and age matched (±10 years and age = 21-year-old cutoff) across groups based on the Spectra MRSA screening assay result (Remel, Thermo Fisher Scientific, Lenexa, Kan): 6 EH+ patients colonized with MSSA, 5 EH+ patients colonized with MRSA, 6 EH+ patients with no *S aureus* colonization, 6 EH– patients colonized with MSSA, 6 EH− patients colonized with MRSA, 6 EH− patients with no *S aureus* colonization, and 5 nonatopic subjects with no *S aureus* colonization and 1 nonatopic subject colonized with MSSA. The more accurate Kirby-Bauer assay was then performed, and the results of this assay were used to assign *S aureus* colonization status for analysis (Table I).

The National Jewish Health Institutional Review Board approved this study. Written informed consent was obtained from each participant or from the parent or legal guardian in the case of minors. Participants aged 7 to 17 years provided assent.

Skin taping and storage

Skin tapings were collected from lesional (mostly chronic/>3 days old) and nonlesional sites of patients with AD and from nonlesional skin of nonatopic subjects, as described previously.[14] Samples were "heat killed" in a water bath at 70°C for 30 minutes to eliminate the risk of infectivity and then frozen in a −80°C freezer. Lack of colony growth on blood agar plates was confirmed in preliminary test samples subjected to 70°C for 30 minutes.

Protein extraction

Proteins were removed from the tape discs with an extraction buffer containing 0.01% 3-(3-[1,1-bisalkyloxyethyl]pyridin-1-yl)propane-1-sulfonate.[14] Extracts from tape discs corresponding to layers 1 to 5, 6 to 10, 11 to 15, and 16 to 20 were combined and processed as previously outlined.[14]

Protein digestion

Proteins were digested as previously described[14] and then purified with Oasis HLB μElution Plate (30 μm) and equipped with a vacuum manifold, according to the manufacturer's directions (Waters, Milford, Mass).

Mass spectrometry

Liquid chromatography and mass spectrometry were carried out as previously described.[14] Samples were run in triplicate on an Agilent 1200 series HPLC (Agilent Technologies, Santa Clara, Calif) and Agilent ETD ion trap (model 6340) mass spectrometer with an HPLC chip.

Database searching

Raw data were extracted and analyzed by using the Spectrum Mill database searching program (Rev A.03.03.080 SR1, Agilent Technologies), as previously described.[14] Data were searched against the SwissProt *Homo sapiens* database (UniProt Release 14).[14] Data were validated, and protein identifications were considered significant if the following confidence thresholds were met: minimum of 2 peptides per protein, protein score greater than 11, individual peptide scores of at least 7, and scored percentage intensity of at least 70%.

Protein selection

Protein database search results were compiled for the triplicate MS runs, pooled layers, subjects, and lesional/nonlesional sites through spectral counting by using an in-house developed Java application (Sun Microsystems, Santa Clara, Calif). Spectral counts were calculated as the sum of the spectra matched to peptides corresponding to a protein in the database to quantify relative protein amounts. Spectral counts were then normalized to the total number of spectra per MS run. Proteins were considered for statistical analysis only if they were present in 2 of 3 technical replicates and if there were at least 6 non-0 values across treatment groups. Spectral counts for each selected protein were averaged across technical replicates and across pooled layers yielding 1 mean spectral count per tape-stripping site (lesional/nonlesional sites for patients with AD and nonlesional sites for nonatopic subjects). All keratins were excluded from statistical analysis because of high homology, which rendered it impossible to distinguish isoforms with confidence.

Statistical methods

Descriptive statistics are presented to characterize all subjects included in the analysis. Categorical data are presented as enumerations and percentages. Continuous data are presented as arithmetic means ± SDs or as medians (25th-75th percentiles) if the distribution of the data is skewed.

Normalized mean spectral counts were modeled by using a linear mixed model with random intercepts to account for the correlation of multiple samples (lesional/nonlesional) for a single subject to compare protein levels between diagnostic groups. The predictors of interest were diagnostic group, *S aureus* colonization status (as measured by using the Kirby-Bauer assay), and sample type, but age and sex were included in the model to account for

TABLE I. Demographics, *S aureus* colonization status, and measures of disease severity by diagnostic group

	EH– patients (n = 18)	EH+ patients (n = 17)	Nonatopic subjects (n = 6)
Age (y)	30.6 ± 18.4	22.0 ± 16.1	30.7 ± 14.1
Sex, no. (%)			
Male	11 (61)	8 (47)	2 (33)
Race, no. (%)			
White	15 (83)	12 (71)	6 (100)
Black	2 (11)	4 (24)	0 (0)
Asian	1 (6)	1 (6)	0 (0)
Ethnicity, no. (%)			
Hispanic	0 (0)	2 (12)	0 (0)
S aureus colonization, no. (%)*			
MRSA	5 (28)	3 (18)	0 (0)
MSSA	7 (39)	8 (47)	1 (17)
No *S aureus*	6 (33)	6 (35)	5 (83)
EASI score†‡	19.6 ± 12.6	16.0 ± 9.4	—
Rajka-Langeland score†‡	7.4 ± 1.2	6.7 ± 1.4	—
Total IgE (KIU/L)§‖	189.0 (52.8-3,680.0)	1,479.0 (409.0-2,949.0)	17.1 (12.0-22.2)

Age, sex, race, ethnicity, *S aureus* colonization status, eczema severity as noted by EASI and Rajka-Langeland scores, and total IgE level according to diagnostic category are shown.

**S aureus* colonization status is based on results of the Kirby-Bauer assay.

†Values presented are the mean ± 1 SD.

‡EASI and Rajka-Langeland scores were assessed for atopic subjects only.

§Total IgE levels were available for 15 EH– patients, 11 EH+ patients, and 2 nonatopic subjects.

‖Values presented are the median (25th-75th percentile).

the study design. Comparisons between diagnostic groups within sample type (EH− lesional vs EH+ lesional, EH− nonlesional vs EH+ nonlesional, EH− nonlesional vs nonatopic nonlesional, and EH+ nonlesional vs nonatopic nonlesional sites) and between sample types within diagnostic groups (EH− lesional vs EH− nonlesional and EH+ lesional vs EH+ nonlesional sites) were made based on model-based estimates of normalized mean spectral count differences and corresponding *P* values. For patients with AD only, similar analyses were also performed on MRSA lesional versus MSSA lesional, MRSA nonlesional versus MSSA nonlesional, MRSA lesional versus no *S aureus* lesional, MRSA nonlesional versus no *S aureus* nonlesional, MSSA lesional versus no *S aureus* lesional, and MSSA nonlesional versus no *S aureus* nonlesional sites. The Benjamini-Hochberg method was used to control the false discovery rate to account for multiple comparisons. Comparisons with a *P* value of less than .0040 were considered significant to control the false discovery rate at a level of 0.05 for the EH analysis. Likewise, for the *S aureus* analysis, comparisons with a *P* value of less than .0005 were considered significant. However, for exploratory purposes, any comparison with a *P* value of less than .05 was considered to be of interest.

The model was expanded to control for severity (Eczema Area and Severity Index [EASI] and Rajka-Langeland scores) and total IgE level to examine the effect of other covariates on the EH comparisons of interest. A sensitivity analysis for the potential effect of race was conducted, in which the EH comparisons were repeated excluding all black subjects.

RESULTS

Demographics, *S aureus* colonization status, and measures of disease severity

Table I presents descriptive statistics characterizing the study sample by diagnostic group. Samples were analyzed from 18 EH− patients (5 with MRSA, 7 with MSSA, and 6 with no *S aureus*), 17 EH+ patients (3 with MRSA, 8 with MSSA, and 6 with no *S aureus*), and 6 nonatopic control subjects (1 with MSSA and 5 with no *S aureus*). Table E1 (available in this article's Online Repository at www.jacionline.org) lists the body locations of skin tapings by diagnostic group.

Complete list of identified proteins

One hundred fifty-three proteins were identified in 2 of 3 technical replicates in layers 1 to 5, 6 to 10, 11 to 15, or 16 to 20 for at least 1 biological sample (see Table E2 in this article's Online Repository at www.jacionline.org). Proteins identified included blood proteins, keratins, skin barrier proteins, and immune-related proteins, among others.

Statistical analysis of protein levels between EH diagnostic groups and sample types

Seventy-one proteins were analyzed for differences in protein expression between diagnostic groups and sample types (see Table E3 in this article's Online Repository at www.jacionline.org). Model-based estimates of normalized mean spectral count differences and corresponding *P* values are presented for each comparison. Comparisons highlighted in gray met the experiment-wise threshold for statistical significance ($P \leq .004$), whereas comparisons with an asterisk did not meet the threshold but were of interest for exploratory purposes. These results are also presented in Table II for proteins related to the skin barrier and generation of natural moisturizing factor (NMF). Again, comparisons highlighted in gray met the experiment-wise threshold for statistical significance ($P \leq .004$), whereas comparisons with a superscript did not meet the threshold but were of interest for exploratory purposes.

Vertical scatter plots of normalized mean spectral count data are presented by diagnostic category and sample type in Figs 1 and 2 for proteins related to the skin barrier and generation of NMF. Statistically significant comparisons are denoted by bars. Arginase-1 was expressed at significantly lower levels in lesional versus nonlesional sites in EH− patients (Fig 1). Bleomycin hydrolase was expressed at lower levels in lesional versus nonlesional sites in both EH− and EH+ patients; however, the difference was not statistically significant (Fig 1). Caspase-14 and protein-glutamine gamma-glutamyltransferase E (also known as transglutaminase 3 [TG3]) were expressed at significantly lower levels in lesional versus nonlesional sites in both EH− and EH+ patients (Fig 1). Filaggrin-2 (flg-2) was expressed at significantly lower levels in lesional versus nonlesional sites in EH− patients and in EH+/EH− nonlesional versus nonatopic sites (Fig 1). Gamma-glutamyl cyclotranserase (GGCT) was expressed at significantly lower levels in lesional versus nonlesional sites in both EH+ and EH– patients (Fig 1). Corneodesmosin and desmocollin-1 were also expressed at significantly lower levels in lesional versus nonlesional sites in both EH– and EH+ patients (Fig 2). Desmoglein-1 was expressed at significantly lower levels in lesional versus nonlesional sites in EH– patients and was expressed at lower levels in EH+ patients; however, the difference in EH+ patients was not statistically significant (Fig 2).

The results of these comparisons did not change appreciably when controlling for severity (as measured based on EASI and

TABLE II. Comparisons of protein expression between EH diagnostic groups and sample types for proteins related to the skin barrier and generation of NMF

Accession no.	Protein name	EH– lesional vs EH+ lesional sites	EH– NL vs EH+ NL sites	EH– NL vs nonatopic NL sites	EH+ NL vs nonatopic NL sites	EH– lesional vs EH– NL sites	EH+ lesional vs EH+ NL sites
P05089	Arginase-1	0.35	4.05	3.55	−0.50	−16.49	−12.79*
Q13867	Bleomycin hydrolase	−2.41	−2.52	−4.24	−1.72	−3.93*	−4.05*
P31944	Caspase-14	−3.09	−0.51	−0.65	−0.14	−23.54	−20.97
Q15517	Corneodesmosin	−0.00	1.18	5.39	4.21	−7.68	−6.51
Q08554	Desmocollin-1	−0.46	−3.29	14.65	17.94*	−18.02	−20.85
Q02413	Desmoglein-1	−2.07	2.68	15.53	12.85	−24.74	−20.00†
Q5D862	flg-2	0.20	7.51*	−15.67	−23.19	−10.63	−3.31
O75223	GGCT	3.55	6.39	14.43‡	8.04	−12.86	−10.02
Q08188	Protein-glutamine gamma-glutamyltransferase E	−2.64	−6.48	−6.10	0.39	−11.07	−14.91

Model-based estimates of normalized mean spectral count difference are shown. A positive mean difference estimate indicates an increase in the first group versus the second group. Comparisons highlighted in gray met the Benjamini-Hochberg threshold for statistical significance (P value threshold = .0040), and comparisons with footnote symbols did not meet the threshold but are of interest.

NL, Nonlesional.

*P = .02.

†P = 5.3E-03.

‡P = .01.

Rajka-Langeland scores) or total IgE level or when excluding black subjects from analysis (data not shown).

Statistical analysis of protein levels between *S aureus* diagnostic groups

Model-based estimates of normalized mean spectral count differences and corresponding P values are presented in Table E4 (available in this article's Online Repository at www.jacionline.org) for each *S aureus* comparison. Epidermal fatty acid–binding protein (e-fabp) was expressed at significantly higher levels in MRSA lesional versus MSSA lesional (50.16 mean spectra, P = 2.4E-04) and MRSA lesional versus no *S aureus* lesional sites (55.65 mean spectra, P = 1.3E-04). Vertical scatter plots of normalized mean spectral count data are presented by diagnostic category and sample type in Fig 3 for e-fabp. Statistically significant comparisons are denoted by bars.

DISCUSSION

These studies reveal decreased levels of proteins related to the skin barrier (flg-2, corneodesmosin, desmoglein-1, desmocollin-1, and transglutaminase-3 [TG3]) and generation of NMF (arginase-1, caspase-14, and GGCT) in lesional versus nonlesional sites of EH+ and EH− patients. Epidermal fatty acid–binding protein was expressed at significantly higher levels in patients with MRSA compared with that seen in patients with MSSA. No significant differences were found between patients with AD with and without a history of EH, but many proteins neared the significance threshold, notably flg-2.

Recent studies indicate that defects in skin barrier proteins are highly associated with the development of AD. Loss-of-function mutations in filaggrin can be found in approximately 20% of patients with AD.[15] Filaggrin, a member of the fused S100 family of S100 Ca^{2+}-binding proteins, is synthesized in the granular layer as a large 400-kd precursor termed profilaggrin.[16] Profilaggrin is stored within keratohyalin granules in the granular layer[17]; as calcium levels increase during differentiation, it undergoes extensive processing, including dephosphorylation and cleavage into filaggrin monomers.[17] In the cornified layer transglutaminases cross-link filaggrin to keratins 1 and 10 to form the insoluble keratin matrix crucial to the development of the skin barrier (Fig 4).[18] Next, the cross-linked filaggrin monomers undergo further posttranslation modification (deimination/citrullination) through the calcium-dependent enzyme peptidylarginine deiminase.[18] This deimination results in disruption of the filaggrin/keratin cross-linking, setting the stage for filaggrin degradation into NMF. NMF refers to a mixture of primarily filaggrin-derived hygroscopic amino acids, including arginine, glutamine, and histidine, and their derivatives citrulline/urea, 2-pyrrolidone-5-carboxylic acid (PCA), and urocanic acid, respectively (Fig 4).[3]

Numerous enzymes are involved in the processing of profilaggrin to filaggrin to NMF, as reviewed by Sandilands et al[17] and Candi et al.[18] In this exploratory study 3 filaggrin/NMF processing enzymes were found to be expressed at significantly lower levels in EH+ and/or EH– lesional AD skin compared with levels seen in nonlesional AD skin (caspase-14, GGCT, and arginase-1). Additionally, bleomycin hydrolase, although not statistically significant, showed a trend toward lower expression in EH+/EH– lesional versus nonlesional skin. Caspase-14 is an enzyme required for the processing of deiminated filaggrin, and homozygous null mice lacking caspase-14 display mild barrier defects characterized by increased transepidermal water loss, decreased stratum corneum hydration, and abnormal filaggrin degradation.[19] The neutral cysteine protease bleomycin hydrolase is important in the final breakdown of partially processed and deiminated filaggrin peptides into amino acids, which are components of the NMF.[20] GGCT catalyzes the formation of pyroglutamic acid or PCA, which is the most abundant NMF found in the stratum corneum.[21] Arginase-1, an enzyme in the urea cycle, hydrolyzes L-arginine into L-ornithine and urea.[22] Arginine is a significant amino acid component of filaggrin[18] and is released on filaggrin degradation. Decreased expression of arginase-1 might decrease urea generation, a hygroscopic component of the NMF.[23] As a whole, these data indicate altered filaggrin processing in lesional skin, which might further exacerbate the

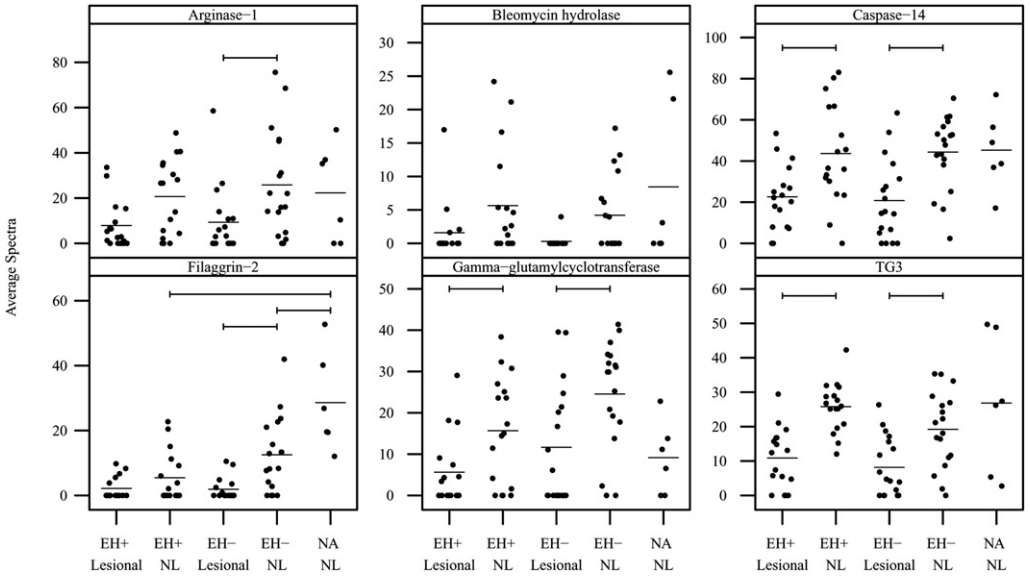

FIG 1. Mean spectral count by diagnostic group and sample type for proteins related to generation of NMF. Vertical scatter plots of the mean spectral count for all subjects by diagnostic group and sample type are shown. *Horizontal bars* denote group means. *Horizontal brackets* denote significant differences between groups (*P* value threshold = .0040). *NL,* Nonlesional.

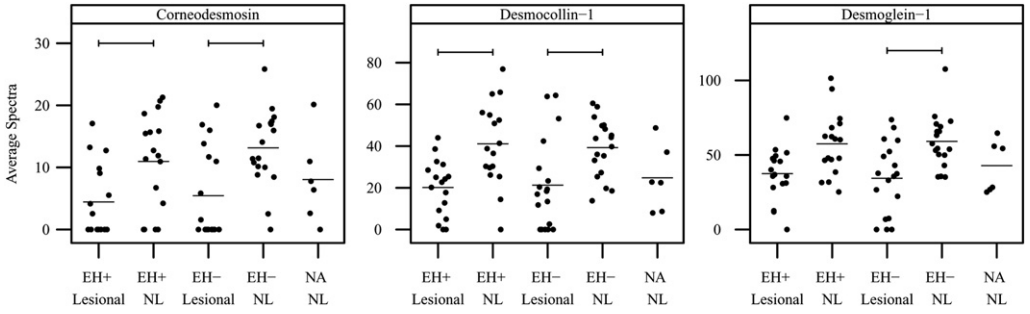

FIG 2. Mean spectral count by diagnostic group and sample type for proteins related to the skin barrier. Vertical scatter plots of the mean spectral count for all subjects by diagnostic group and sample type are shown. *Horizontal bars* denote group means. *Horizontal brackets* denote significant differences between groups (*P* value threshold = .0040). *NL,* Nonlesional.

disease process through abnormal corneocyte development, and a decrease in the amount of NMF, which is crucial to skin hydration. Restoration of NMF components with creams and moisturizers containing urea or PCA has been shown to alleviate the symptoms of AD, reduce the risk of relapse, or both, as reviewed by Loden.[24] The ability of these NMF-based creams to restore the skin barrier further highlights the critical role of NMFs in skin barrier integrity.

In addition to lower expression of enzymes involved in NMF generation, our current work revealed lower expression of 3 proteins directly linked to the skin barrier and corneodesmosome structure. Corneodesmosomes, comprised of desmoglein-1, desmocollin-1, and corneodesmosin, bind keratins to the cellular membrane and serve to tightly attach adjacent corneocytes (Fig 4).[18] Through a tightly controlled process, corneodesmosomes are proteolytically degraded in the uppermost layers of the stratum corneum to allow desquamation. The remaining keratins are covalently attached to the cell envelope and provide mechanical resistance.[25] Simultaneously, the cytosolic enzyme TG3 mediates the cross-linking of loricrin to small proline-rich proteins; this complex further reinforces the cell membrane (Fig 4). The significantly lower expression of desmocollin-1, desmoglein-1, corneodesmosin, and TG3 in lesional skin could be indicative of inappropriate desquamation[26] or abnormal differentiation,[3] both of which have been found in patients with AD.[13]

flg-2 is one of 5 genes in the S100 fused-type protein gene cluster.[27,28] It is closely homologous to filaggrin, but the precise role of flg-2 in skin biology is unknown. The lower expression of flg-2 in both EH+/EH– nonlesional skin versus nonatopic nonlesional skin and in EH– lesional versus nonlesional skin indicate a potential role in maintenance of the skin barrier. Furthermore, there was a trend toward lower levels of flg-2 in EH+ nonlesional compared with EH– nonlesional skin ($P = .02$). Unpublished work reported in a patent by Schroder (patent pending) indicates potent antimicrobial activity of the C-terminal of flg-2 against the soil bacterium *Pseudomonas* species. Our

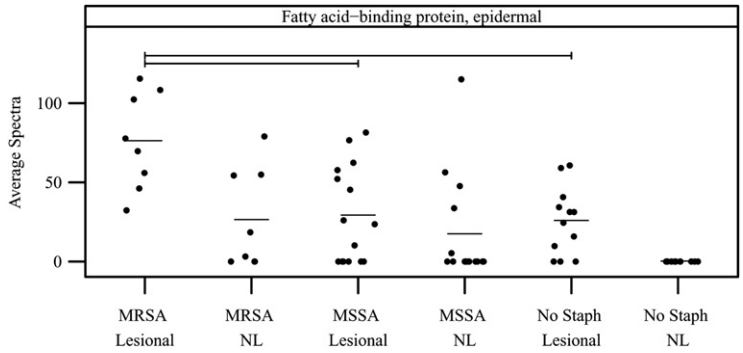

FIG 3. Mean spectral count by *S aureus* infection group and sample type for e-fabp. Vertical scatter plots of the mean spectral count for all subjects by *S aureus* infection group and sample type are shown. *Horizontal bars* denote group means. *Horizontal brackets* denote significant differences between groups (*P* value threshold = .0005). *NL*, Nonlesional.

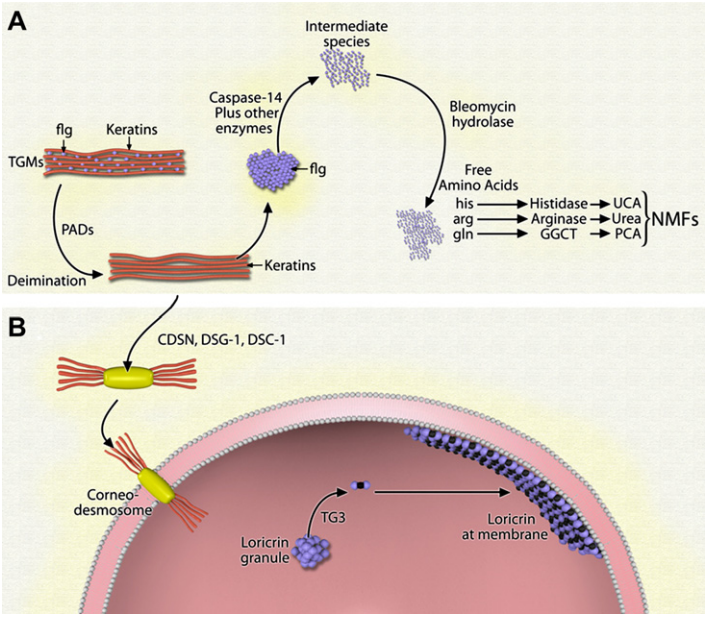

FIG 4. **A**, Generation of NMF. In the cornified layer transglutaminases *(TGMs)* cross-link filaggrin *(flg)* to keratins 1 and 10 to form keratin bundles. The cross-linked filaggrin monomers undergo further posttranslational modification (deimination) through the calcium-dependent enzyme peptidylarginine deiminase *(PAD)*. Deiminated filaggrin is released from the complex and further processed by caspase-14 and other enzymes into an intermediate species, which is further processed by bleomycin hydrolase into free amino acids (arg, his, and gln). Arginine (arg) is processed by arginase-1 to urea, glutamine (gln) is processed by GGCT to PCA, and histidine (his) is processed by histidase into urocanic acid. NMF refers to this mixture of primarily filaggrin-derived hygroscopic amino acids and their derivatives. **B**, Corneodesmosome formation and reinforcement of corneocyte membrane. On release of filaggrin monomers, keratins bind to desmoglein-1, desmocollin-1, and corneodesmosin to form corneodesmosomes, which tightly bind adjacent corneocytes. Simultaneously, TG3 binds loricrin to small proline-rich proteins, and this complex further reinforces the inner membrane of the corneocyte.

observed lower expression of flg-2 in EH+ nonlesional skin is consistent with previous work showing an association of impaired skin barrier and decreased antimicrobial activity in subjects prone to EH.[29,30] Further work needs to be done on this potentially important skin barrier protein and antimicrobial peptide as a biomarker distinguishing EH+ versus EH– patients.

In our present study e-fabp levels were increased in MRSA lesional versus MSSA lesional and MRSA lesional versus no *S aureus* lesional sites. Increased levels of fatty acid–binding protein in patients with AD have been reported in other studies,[14,31] but none evaluated concurrent *S aureus* colonization status. Fatty acid–binding proteins are abundant intracellular proteins that bind and transport otherwise insoluble long-chain fatty acids.[32] Epidermal fatty acid–binding protein is found in the basal and granular cell layers in normal human skin[33] and appears to be essential for normal keratinocyte differentiation.[34] It has been proposed that fatty acid–binding proteins might serve as master regulators of inflammatory and metabolic signaling pathways.[35] In support of this, e-fabp has been shown to bind and stabilize leukotriene A_4 and might modulate the production or metabolism of

bioactive eicosanoids, which have been found in the urine of patients with AD.[36,37] In addition to a potential role in eicosanoid signaling, e-fabp might also serve as an antioxidant protein and has been shown to bind 4-hydroxynonenal, a highly reactive aldehyde byproduct of lipid peroxidation.[38] Increased levels of urinary oxidative stress markers have been found in patients with AD,[39] as well as direct evidence of oxidative stress in the stratum corneum in patients with AD.[40] It is possible that oxidative stress might induce e-fabp, and this might be exacerbated in patients with AD with MRSA infection. Additionally, liver fatty acid–binding protein has been shown to be a critical host factor for malaria[41] and increases the intracellular growth of chlamydia.[42] Further studies are needed to determine whether e-fabp might promote *S aureus* growth or serve as a host factor for infection.

In conclusion, we have found lower expression of skin barrier proteins in lesional skin of patients with AD. These proteins are involved in the generation of the NMF, corneodesmosomes, and antimicrobial host defense. These changes might reflect defective differentiation of corneocytes in patients with AD and promote susceptibility to skin infection. The findings presented here support recent work highlighting broad defects in epidermal cornification in patients with AD.[13] In addition, increased e-fabp levels in MRSA-infected patients with AD might indicate aberrant eicosanoid signaling or oxidative stress, or e-fabp might serve as a host factor for *S aureus* colonization.

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Key messages

- **The lower levels of 4 enzymes involved in the generation of the NMF in lesional AD skin are a novel finding. This defect could perpetuate the dry skin cycle and predispose patients to infection.**
- **The lower levels of key skin barrier proteins involved in the generation of corneodesmosomes might result in decreased corneocyte adhesion. The lower levels of flg-2 in AD lesions might indicate a skin barrier defect or a decrease in antimicrobial peptides**
- **The higher levels of e-fabp in patients colonized with MRSA compared with those seen in patients colonized with MSSA or no S *aureus* might represent a protective mechanism to increased oxidative stress or might perpetuate the inflammatory response.**

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TABLE E1. Distribution of skin tapings by body location

	EH– patients (n = 18)	EH+ patients (n = 17)	Nonatopic subjects (n = 6)
Nonlesional tapings			
Arm	13 (72%)	12 (71%)	6 (100%)
Leg	4 (22%)	4 (24%)	0 (0%)
Torso	1 (6%)	1 (6%)	0 (0%)
Lesional tapings			
Arm	12 (67%)	12 (71%)	
Leg	4 (22%)	4 (24%)	
Torso	2 (11%)	1 (6%)	

TABLE E2. Complete list of proteins identified

Swiss Prot accession no.	Protein name
P02768	Serum albumin
P08779	Keratin, type I cytoskeletal 16
P01023	Alpha-2-macroglobulin
P01857	Ig gamma-1 chain C region
P04264	Keratin, type II cytoskeletal 1
P02538	Keratin, type II cytoskeletal 6A
P02787	Serotransferrin
P00738	Haptoglobin
P01842	Ig lambda chain C regions
P29508	Serpin B3
P06733	Alpha-enolase
P01009	Alpha-1-antitrypsin
Q01469	Fatty acid-binding protein, epidermal
P60709	Actin cytoplasmic 1
P68871	Hemoglobin subunit beta
P01834	Ig kappa chain C region
P01024	Complement C3
P02679	Fibrinogen gamma chain
P02675	Fibrinogen beta chain
P01876	Ig alpha-1 chain C region
P06702	Protein S100-A9
P02774	Vitamin D-binding protein
P48668	Keratin type II cytoskeletal 6C
P69905	Hemoglobin subunit alpha
P02647	Apolipoprotein A-I
P59665	Neutrophil defensin 1
Q6S8J3	POTE ankyrin domain family member E
P48594	Serpin B4
Q02413	Desmoglein-1
P07355	Annexin A2
P13645	Keratin type I cytoskeletal 10
P10599	Thioredoxin
P25311	Zinc-alpha-2-glycoprotein
P13639	Elongation factor 2
Q08554	Desmocollin-1
P01861	Ig gamma-4 chain C region
P31944	Caspase-14
P35908	Keratin, type II cytoskeletal 2 epidermal
P04406	Glyceraldehyde-3-phosphate dehydrogenase
Q08188	Protein-glutamine gamma-glutamyltransferase E
P07339	Cathepsin D
P31151	Protein S100-A7
P05089	Arginase-1
P19971	Thymidine phosphorylase
P60174	Triosephosphate isomerase
Q14574	Desmocollin-3
Q15517	Corneodesmosin
O75223	Gamma-glutamyl cyclotransferase
Q15828	Cystatin-M
Q9NZT1	Calmodulin-like protein 5
Q06830	Peroxiredoxin-1
P05109	Protein S100-A8
P04075	Fructose-bisphosphate aldolase A
Q9NZH8	Interleukin-1 family member 9
P15924	Desmoplakin
P63104	14-3-3 protein zeta/delta
P47929	Galectin-7
P31947	14-3-3 protein sigma
P68032	Actin alpha cardiac muscle 1
P00558	Phosphoglycerate kinase 1
P13647	Keratin, type II cytoskeletal 5
P32119	Peroxiredoxin-2
Q96P63	Serpin B12
P14618	Pyruvate kinase isozymes M1/M2
Q5D862	Filaggrin-2
Q53RT3	Retroviral-like aspartic protease 1
P27482	Calmodulin-like protein 3
Q6E0U4	Dermokine
P36952	Serpin B5
P00338	L-lactate dehydrogenase A chain
P04792	Heat shock protein beta-1
P35754	Glutaredoxin-1
P09211	Glutathione S-transferase P
Q13867	Bleomycin hydrolase
P50395	Rab GDP dissociation inhibitor beta
P56537	Eukaryotic translation initiation factor 6
P06396	Gelsolin
P31949	Protein S100-A11
Q6UWP8	Suprabasin
A8K2U0	Alpha-2-macroglobulin-like protein 1
P04040	Catalase
P11021	78-kd Glucose-regulated protein
P63261	Actin cytoplasmic 2
P31025	Lipocalin-1
P02533	Keratin, type I cytoskeletal 14
P20930	Filaggrin
P04220	Ig mu heavy chain disease protein
P01871	Ig mu chain C region
P08107	Heat shock 70-kd protein 1
P22531	Small proline-rich protein 2E
P06744	Glucose-6-phosphate isomerase
Q9UI42	Carboxypeptidase A4
P54652	Heat shock-related 70-kd protein 2
P62158	Calmodulin
P11142	Heat shock cognate 71-kd protein
Q04695	Keratin, type I cytoskeletal 17
P62937	Peptidyl-prolyl cis-trans isomerase A
P62736	Actin aortic smooth muscle
P02765	Alpha-2-HS-glycoprotein
P01877	Ig alpha-2 chain C region
P01859	Ig gamma-2 chain C region
P13284	Gamma-interferon-inducible lysosomal thiol reductase
P81605	Dermcidin
P00441	Superoxide dismutase [Cu-Zn]
P07384	Calpain-1 catalytic subunit
Q7Z794	Keratin, type II cytoskeletal 1b
Q99497	Protein DJ-1
O43790	Keratin type II cuticular Hb6
P50452	Serpin B8
P35237	Serpin B6
Q15323	Keratin type I cuticular Ha1
Q92820	Gamma-glutamyl hydrolase
P00915	Carbonic anhydrase 1
P17900	Ganglioside GM2 activator
P35527	Keratin, type I cytoskeletal 9
O95274	Ly6/PLAUR domain-containing protein 3
P13796	Plastin-2
Q8WWY7	WAP four-disulfide core domain protein 12
Q99880	Histone H2B type 1-L
P04083	Annexin A1
P02788	Lactotransferrin
P07737	Profilin-1

(Continued)

TABLE E2. *(Continued)*

(Continued)

TABLE E2. *(Continued)*

Swiss Prot accession no.	Protein name
P35326	Small proline-rich protein 2A
Q9UGL9	Cysteine-rich C-terminal protein 1
P23528	Cofilin-1
P80188	Neutrophil gelatinase-associated lipocalin
P01860	Ig gamma-3 chain C region
P04259	Keratin type II cytoskeletal 6B
P55000	Secreted Ly-6/uPAR-related protein 1
P14735	Insulin-degrading enzyme
P12814	Alpha-actinin-1
P01040	Cystatin-A
Q86SR0	Secreted Ly-6/uPAR-related protein 2
P09668	Cathepsin H
P61970	Nuclear transport factor 2
P12273	Prolactin-inducible protein
Q14525	Keratin type I cuticular Ha3-II
O75635	Serpin B7
P48163	NADP-dependent malic enzyme
P23490	Loricrin
Q86SG5	Protein S100-A7A
Q9H0W9	Ester hydrolase C11orf54
P15531	Nucleoside diphosphate kinase A
O60361	Putative nucleoside diphosphate kinase
P04217	Alpha-1B-glycoprotein
P02671	Fibrinogen alpha chain
P13929	Beta-enolase
P02766	Transthyretin
P61916	Epididymal secretory protein E1
P10909	Clusterin
P00739	Haptoglobin-related protein
P01036	Cystatin-S
P20933	N(4)-(beta-N-acetylglucosaminyl)-L-asparaginase
P61626	Lysozyme C

TABLE E3. Comparisons of protein expression, as measured by normalized mean spectral count, between EH diagnostic groups and sample type

Accession no.	Protein name	EH– lesional vs EH+ lesional sites	EH– NL vs EH+ NL sites	EH– NL vs nonatopic NL sites	EH+ NL vs nonatopic NL sites	EH– lesional vs EH– NL sites	EH+ lesional vs EH+ NL sites
P31947	14-3-3 protein sigma						
	Estimate	−2.45	−1.81	0.69	2.50	2.67	3.31
	P value	.35	.49	.85	.48	.23	.15
P68032	Actin alpha cardiac muscle 1						
	Estimate	−0.82	0.04	0.70	0.66	1.56	2.42
	P value	.55	.98	.71	.73	.21	.07
P60709	Actin cytoplasmic 1						
	Estimate	−0.19	−0.76	0.33	1.09	5.65*	5.08*
	P value	.94	.75	.92	.74	.02*	.04*
P01009	Alpha-1-antitrypsin						
	Estimate	−4.86	0.38	0.94	0.56	7.75*	12.98
	P value	.20	.92	.86	.91	.04*	1.2E-03
P01023	Alpha-2-macroglobulin						
	Estimate	0.07	0.60	−0.30	−0.89	5.45	5.97
	P value	.98	.85	.94	.84	.08	.06
A8K2U0	Alpha-2-macroglobulin–like protein 1						
	Estimate	−0.20	−0.34	1.42	1.76	−0.97	−1.11
	P value	.84	.73	.30	.20	.31	.26
P06733	Alpha-enolase						
	Estimate	−8.48	−11.77	9.79	21.56	17.43*	14.14
	P value	.33	.18	.41	.08	.02*	.06
P07355	Annexin A2						
	Estimate	−5.59	5.69	16.18	10.49	−28.02	−16.75*
	P value	.45	.44	.12	.30	3.8E-04	.03*
P02647	Apolipoprotein A-I						
	Estimate	1.88	−0.17	−0.02	0.14	3.15*	1.11
	P value	.13	.89	.99	.93	9.5E-03	.35
P05089	Arginase-1						
	Estimate	0.35	4.05	3.55	−0.50	−16.49	−12.79*
	P value	.96	.53	.69	.95	3.4E-03	.02*
Q13867	Bleomycin hydrolase						
	Estimate	−2.41	−2.52	−4.24	−1.72	−3.93*	−4.05*
	P value	.27	.25	.16	.56	.02*	.02*
P27482	Calmodulin-like protein 3						
	Estimate	−1.17	−0.81	0.11	0.92	0.90	1.26
	P value	.25	.43	.94	.51	.27	.14
Q9NZT1	Calmodulin-like protein 5						
	Estimate	−0.21	−2.80	3.32	6.13	6.98	4.39
	P value	.96	.55	.60	.34	.09	.29
Q9UI42	Carboxypeptidase A4						
	Estimate	−0.87	0.20	1.02	0.83	−1.27	−0.20
	P value	.22	.78	.29	.40	.07	.77
P31944	Caspase-14						
	Estimate	−3.09	−0.51	−0.65	−0.14	−23.54	−20.97
	P value	.64	.94	.94	.99	1.8E-04	8.5E-04
P04040	Catalase						
	Estimate	−0.27	1.71	−3.62	−5.33	−9.06	−7.08*
	P value	.93	.58	.39	.22	1.7E-03	.01*
P07339	Cathepsin D						
	Estimate	0.35	−2.07	10.49	12.56	−7.61*	−10.03*
	P value	.95	.71	.17	.11	.05*	.01*
P09668	Cathepsin H						
	Estimate	0.17	2.07*	1.81	−0.25	−2.00*	−0.10
	P value	.87	.04*	.18	.85	.03*	.91
P01024	Complement C3						
	Estimate	1.65	0.21	0.08	−0.13	3.06*	1.63
	P value	.22	.87	.97	.94	.02*	.22

(Continued)

TABLE E3. *(Continued)*

Accession no.	Protein name	EH– lesional vs EH+ lesional sites	EH– NL vs EH+ NL sites	EH– NL vs nonatopic NL sites	EH+ NL vs nonatopic NL sites	EH– lesional vs EH– NL sites	EH+ lesional vs EH+ NL sites
Q15517	Corneodesmosin						
	Estimate	−0.00	1.18	5.39	4.21	−7.68	−6.51
	P value	1.00	.62	.10	.20	2.7E-05	3.1E-04
P01040	Cystatin-A						
	Estimate	0.45	0.11	1.41	1.30	−0.89	−1.23
	P value	.50	.87	.13	.17	.16	.06
Q15828	Cystatin-M						
	Estimate	−0.14	−4.45	5.06	9.52*	−0.53	−4.84*
	P value	.95	.06	.11	4.6E-03	.74	6.5E-03
P81605	Dermcidin						
	Estimate	1.70	−1.35	−5.78	−4.43	−1.81	−4.86*
	P value	.57	.65	.16	.28	.43	.05*
Q6E0U4	Dermokine						
	Estimate	0.45	−1.58	1.17	2.75	0.56	−1.47
	P value	.66	.13	.41	.06	.49	.09
Q08554	Desmocollin-1						
	Estimate	−0.46	−3.29	14.65	17.94*	−18.02	−20.85
	P value	.93	.55	.06	.02*	2.9E-04	7.1E-05
Q14574	Desmocollin-3						
	Estimate	−1.67	−2.80	1.59	4.39	−0.75	−1.88
	P value	.36	.13	.52	.08	.43	.06
Q02413	Desmoglein-1						
	Estimate	−2.07	2.68	15.53	12.85	−24.74	−20.00*
	P value	.78	.71	.13	.21	6.2E-04	5.3E-03
P15924	Desmoplakin						
	Estimate	0.66	−0.32	2.06	2.37	1.59	0.62
	P value	.76	.88	.49	.42	.26	.67
P13639	Elongation factor 2						
	Estimate	−2.45	−3.82	1.58	5.40	0.90	−0.47
	P value	.32	.12	.64	.12	.70	.84
P56537	Eukaryotic translation initiation factor 6						
	Estimate	−0.21	−0.48	2.41	2.89	−1.87*	−2.13*
	P value	.85	.68	.13	.08	.02*	.01*
Q01469	Fatty acid–binding protein, epidermal						
	Estimate	−2.84	−5.89	9.56	15.44	26.75*	23.71*
	P value	.76	.52	.45	.23	4.4E-03	.01*
P20930	Filaggrin						
	Estimate	−0.26	−1.15	−5.63*	−4.49	0.76	−0.12
	P value	.88	.52	.02*	.07	.23	.85
Q5D862	Fillagrin-2						
	Estimate	0.20	7.51*	−15.67	−23.19	−10.63	−3.31
	P value	.95	.02*	6.9E-04	5.0E-06	1.0E-04	.18
P04075	Fructose-bisphosphate aldolase A						
	Estimate	−0.35	−0.41	0.07	0.48	0.69	0.63
	P value	.58	.51	.94	.58	.24	.30
P47929	Galectin-7						
	Estimate	−1.13	−1.52	0.35	1.87	1.27	0.87
	P value	.44	.30	.86	.35	.21	.40
Q92820	Gamma-glutamyl hydrolase						
	Estimate	0.10	0.62	0.86	0.24	−0.68	−0.17
	P value	.83	.20	.20	.72	.13	.71
O75223	Gamma-glutamyl cyclotransferase						
	Estimate	3.55	6.39	14.43*	8.04	−12.86	−10.02
	P value	.38	.12	.01*	.16	4.4E-05	1.1E-03

(Continued)

TABLE E3. *(Continued)*

Accession no.	Protein name	EH– lesional vs EH+ lesional sites	EH– NL vs EH+ NL sites	EH– NL vs nonatopic NL sites	EH+ NL vs nonatopic NL sites	EH– lesional vs EH– NL sites	EH+ lesional vs EH+ NL sites
P13284	Gamma-interferon–inducible lysosomal thiol reductase						
	Estimate	0.11	1.40*	1.00	−0.40	−1.56	−0.27
	P value	.83	7.9E-03	.15	.55	3.0E-04	.50
P09211	Glutathione S-transferase P						
	Estimate	−0.03	−1.26	−0.06	1.20	1.27	0.05
	P value	.97	.24	.97	.41	.16	.96
P04406	Glyceraldehyde-3-phosphate dehydrogenase						
	Estimate	−0.81	−3.37	3.34	6.71	−0.30	−2.86
	P value	.86	.45	.59	.28	.92	.33
	Haptoglobin						
P00738	Estimate	6.17	1.27	1.28	0.01	8.72*	3.82
	P value	.13	.75	.82	1.00	.03*	.34
P04792	Heat shock protein beta-1						
	Estimate	0.26	−0.01	0.43	0.44	0.19	−0.08
	P value	.57	.98	.49	.49	.66	.85
P69905	Hemoglobin subunit alpha						
	Estimate	−7.88	−1.57	0.83	2.40	2.42	8.73
	P value	3.6E-03	.53	.81	.49	.28	5.9E-04
P68871	Hemoglobin subunit beta						
	Estimate	−10.02*	−1.57	1.38	2.96	6.17	14.62
	P value	.02*	.69	.80	.59	.09	4.1E-04
P01876	Ig alpha-1 chain C region						
	Estimate	0.62	0.15	1.16	1.01	3.85	3.38
	P value	.78	.95	.70	.74	.08	.13
P01857	Ig gamma-1 chain C region						
	Estimate	−1.62	−0.35	5.54	5.89	14.91	16.18
	P value	.74	.94	.40	.38	4.3E-04	2.4E-04
P01834	Ig kappa chain C region						
	Estimate	6.74	3.52	5.55	2.03	19.44	16.22
	P value	.13	.42	.35	.73	4.4E-05	5.3E-04
P01842	Ig lambda chain C regions						
	Estimate	−3.07	0.26	0.58	0.32	10.72*	14.06
	P value	.45	.95	.92	.95	7.9E-03	1.1E-03
Q9NZH8	Interleukin-1 family member 9						
	Estimate	−0.34	−0.44	2.80	3.24*	−1.28	−1.37
	P value	.75	.68	.06	.03*	.17	.15
P00338	L-lactate dehydrogenase A chain						
	Estimate	1.55	−2.06	−0.09	1.96	2.57	−1.04
	P value	.35	.22	.97	.39	.11	.52
P31025	Lipocalin-1						
	Estimate	−0.31	−0.04	3.60	3.64	−3.94	−3.66
	P value	.92	.99	.42	.42	.14	.18
Q6S8J3	POTE ankyrin domain family member E						
	Estimate	1.96	−0.41	−0.04	0.37	2.02	−0.35
	P value	.12	.74	.98	.83	.10	.78
Q06830	Peroxiredoxin-1						
	Estimate	−1.59	−2.83*	0.23	3.06	0.46	−0.78
	P value	.24	.04*	.90	.11	.56	.34
P32119	Peroxiredoxin-2						
	Estimate	1.34	2.69	0.54	−2.15	−2.28	−0.93
	P value	.50	.18	.84	.43	.16	.58
P31151	Protein S100-A7						
	Estimate	2.14	−5.48	14.06	19.53*	1.46	−6.16
	P value	.72	.36	.09	.02*	.71	.13
P05109	Protein S100-A8						
	Estimate	−1.13	−0.08	0.94	1.02	2.14	3.20*
	P value	.55	.96	.71	.69	.06	9.4E-03

(Continued)

TABLE E3. *(Continued)*

Accession no.	Protein name	EH– lesional vs EH+ lesional sites	EH– NL vs EH+ NL sites	EH– NL vs nonatopic NL sites	EH+ NL vs nonatopic NL sites	EH– lesional vs EH– NL sites	EH+ lesional vs EH+ NL sites
P06702	Protein S100-A9						
	Estimate	−0.53	−1.00	0.52	1.52	2.05	1.58
	P value	.73	.51	.80	.47	.08	.18
Q08188	Protein-glutamine gamma-glutamyltransferase E						
	Estimate	−2.64	−6.48	−6.10	0.39	−11.07	−14.91
	P value	.48	.09	.23	.94	6.1E-04	2.3E-05
P14618	Pyruvate kinase isozymes M1/M2						
	Estimate	−2.44	−1.07	0.30	1.37	0.58	1.95
	P value	.08	.44	.87	.47	.60	.09
Q53RT3	Retroviral-like aspartic protease 1						
	Estimate	−0.94	−1.22	0.35	1.57	1.26	0.98
	P value	.35	.23	.80	.26	.09	.19
P02787	Serotransferrin						
	Estimate	9.04	2.80	1.96	−0.84	16.59*	10.36
	P value	.12	.62	.80	.91	4.4E-03	.07
Q96P63	Serpin B12						
	Estimate	3.03	11.20*	−6.90	−18.10*	−21.41	−13.25*
	P value	.57	.04*	.35	.02*	2.6E-05	5.5E-03
P29508	Serpin B3						
	Estimate	−15.54	−10.89	18.21	29.10	19.37	24.02
	P value	.22	.39	.30	.10	.12	.06
P35237	Serpin B6						
	Estimate	0.01	−0.84	0.30	1.14	−0.03	−0.88*
	P value	.98	.10	.66	.11	.94	.02*
P50452	Serpin B8						
	Estimate	0.21	0.52	0.79	0.27	−2.23	−1.91
	P value	.91	.78	.76	.92	.08	.14
P02768	Serum albumin						
	Estimate	49.22	9.12	23.12	14.00	137.33	97.23*
	P value	.22	.82	.67	.80	6.5E-04	.01*
P00441	Superoxide dismutase [Cu-Zn]						
	Estimate	0.62	1.65	1.54	−0.11	−2.21*	−1.18
	P value	.47	.06	.19	.92	6.6E-03	.14
P10599	Thioredoxin						
	Estimate	−11.71	−9.91	4.60	14.52	−11.79*	−9.99
	P value	.28	.36	.75	.33	.05*	.10
P19971	Thymidine phosphorylase						
	Estimate	−3.10	−1.45	0.72	2.17	−0.24	1.41
	P value	.06	.37	.74	.32	.77	.10
P60174	Triosephosphate isomerase						
	Estimate	−1.56	−12.42*	11.64	24.06*	4.94	−5.92
	P value	.80	.05*	.17	7.0E-03	.29	.22
P25311	Zinc-alpha-2-glycoprotein						
	Estimate	8.57	12.68	−0.68	−13.36	−33.44	−29.34
	P value	.31	.14	.95	.25	1.7E-05	1.4E-04

Comparisons highlighted in gray met the experiment-wise threshold for statistical significance ($P \leq .004$), whereas comparisons with an *asterisk* did not meet the threshold but were of interest for exploratory purposes.
NL, Nonlesional.

TABLE E4. Comparisons of protein expression, as measured by normalized mean spectral count, between *S aureus* colonization groups and sample type

Accession no.	Protein name	MRSA lesional vs MSSA lesional sites	MRSA NL vs MSSA NL sites	MRSA lesional vs no *S aureus* lesional sites	MRSA NL vs no *S aureus* NL sites	MSSA lesional vs no *S aureus* lesional sites	MSSA NL vs no *S aureus* NL sites
P31947	14-3-3 protein sigma						
	Estimate	−1.41	−2.48	−0.55	1.44	0.85	3.92
	P value	.68	.47	.88	.69	.78	.20
P68032	Actin alpha cardiac muscle 1						
	Estimate	0.07	−0.55	0.12	0.68	0.06	1.23
	P value	.97	.76	.95	.72	.97	.45
P60709	Actin cytoplasmic 1						
	Estimate	−4.20	−0.12	−8.18*	0.18	−3.98	0.30
	P value	.19	.97	.02*	.96	.17	.92
P01009	Alpha-1-antitrypsin						
	Estimate	−12.61*	−0.17	−4.94	0.90	7.67	1.07
	P value	.02*	.97	.35	.86	.09	.81
P01023	Alpha-2-macroglobulin						
	Estimate	−9.37*	1.00	−1.28	1.02	8.09*	0.02
	P value	.03*	.81	.77	.82	.04*	1.00
A8K2U0	Alpha-2-macroglobulin–like protein 1						
	Estimate	−0.84	−2.27	0.13	−0.27	0.97	2.00
	P value	.52	.09	.92	.84	.41	.09
P06733	Alpha-enolase						
	Estimate	27.50*	11.57	31.01*	22.10	3.51	10.53
	P value	.02*	.31	.01*	.07	.73	.30
P07355	Annexin A2						
	Estimate	23.78*	−9.11	4.32	−9.27	−19.47*	−0.16
	P value	.02*	.33	.66	.35	.02*	.98
P02647	Apolipoprotein A-I						
	Estimate	−3.52*	−0.15	−0.98	−0.24	2.54	−0.09
	P value	.03*	.92	.56	.89	.08	.95
P05089	Arginase-1						
	Estimate	−4.71	−10.77	−6.21	−13.22	−1.50	−2.45
	P value	.56	.18	.46	.12	.83	.73
Q13867	Bleomycin hydrolase						
	Estimate	−2.10	−3.44	−1.34	−1.35	0.76	2.09
	P value	.38	.16	.60	.60	.72	.33
P27482	Calmodulin-like protein 3						
	Estimate	−0.34	1.39	1.44	1.62	1.78	0.23
	P value	.80	.30	.31	.26	.14	.85
Q9NZT1	Calmodulin-like protein 5						
	Estimate	14.53*	1.47	19.03*	6.76	4.50	5.28
	P value	.02*	.81	5.9E-03	.30	.41	.34
Q9UI42	Carboxypeptidase A4						
	Estimate	−0.48	0.44	−1.57	−0.91	−1.10	−1.35
	P value	.60	.63	.11	.35	.19	.11
P31944	Caspase-14						
	Estimate	−2.44	−11.57	−16.98	−26.49*	−14.54	−14.92*
	P value	.77	.17	.06	4.6E-03	.05	.05*
P04040	Catalase						
	Estimate	−3.02	−12.09*	−5.02	−10.31*	−1.99	1.78
	P value	.44	3.8E-03	.23	.02*	.56	.61
P07339	Cathepsin D						
	Estimate	3.17	−8.70	−9.63	−14.99	−12.81	−6.28
	P value	.66	.23	.21	.06	.05	.33
P09668	Cathepsin H						
	Estimate	−0.09	−0.97	−0.13	−1.07	−0.04	−0.10
	P value	.94	.45	.93	.43	.97	.93
P01024	Complement C3						
	Estimate	−3.27	0.14	−0.39	0.29	2.88	0.15
	P value	.07	.94	.83	.88	.07	.92

(Continued)

TABLE E4. *(Continued)*

Accession no.	Protein name	MRSA lesional vs MSSA lesional sites	MRSA NL vs MSSA NL sites	MRSA lesional vs no *S aureus* lesional sites	MRSA NL vs no *S aureus* NL sites	MSSA lesional vs no *S aureus* lesional sites	MSSA NL vs no *S aureus* NL sites
Q15517	Corneodesmosin						
	Estimate	3.51	−1.77	1.47	−5.45	−2.05	−3.68
	P value	.25	.56	.65	.10	.45	.18
P01040	Cystatin-A						
	Estimate	−0.10	−0.19	−0.42	−1.99*	−0.32	−1.80*
	P value	.91	.83	.65	.04*	.68	.03*
Q15828	Cystatin-M						
	Estimate	−0.70	−5.18	−0.91	−2.14	−0.21	3.04
	P value	.81	.09	.77	.50	.94	.26
P81605	Dermcidin						
	Estimate	−1.68	3.38	−0.52	6.44	1.15	3.07
	P value	.66	.38	.90	.12	.73	.37
Q6E0U4	Dermokine						
	Estimate	2.02	−0.61	0.23	−0.16	−1.79	0.46
	P value	.14	.65	.87	.91	.14	.70
Q08554	Desmocollin-1						
	Estimate	13.53	−9.32	−4.31	−14.47	−17.84*	−5.15
	P value	.06	.19	.56	.06	7.1E-03	.41
Q14574	Desmocollin-3						
	Estimate	1.58	2.71	0.08	0.75	−1.51	−1.97
	P value	.51	.26	.98	.77	.48	.36
Q02413	Desmoglein-1						
	Estimate	16.50	12.40	2.30	8.97	−14.20	−3.44
	P value	.09	.19	.82	.37	.10	.68
P15924	Desmoplakin						
	Estimate	0.29	1.00	0.97	−0.44	0.68	−1.44
	P value	.92	.73	.75	.88	.79	.57
P13639	Elongation factor 2						
	Estimate	8.68*	3.63	10.34*	7.86*	1.66	4.23
	P value	.01*	.26	4.5E-03	.03*	.56	.15
P56537	Eukaryotic translation initiation factor 6						
	Estimate	−1.03	0.01	−0.84	−0.51	0.19	−0.52
	P value	.50	1.00	.60	.75	.89	.71
Q01469	Fatty acid–binding protein, epidermal						
	Estimate	50.16	12.21	55.65	31.50*	5.49	19.28
	P value	2.4E-04	.32	1.3E-04	.02*	.61	.08
P20930	Filaggrin						
	Estimate	−1.72	−0.93	0.55	0.55	2.27*	1.48
	P value	.11	.38	.62	.62	.02*	.12
Q5D862	Filaggrin-2						
	Estimate	0.55	−2.37	−2.08	−4.32	−2.63	−1.96
	P value	.87	.50	.57	.24	.40	.53
P04075	Fructose-bisphosphate aldolase A						
	Estimate	1.72*	0.96	1.33	1.03	−0.38	0.07
	P value	.04*	.25	.13	.24	.60	.92
P47929	Galectin-7						
	Estimate	−0.50	−2.04	−1.26	0.51	−0.77	2.54
	P value	.80	.30	.54	.80	.66	.15
Q92820	Gamma-glutamyl hydrolase						
	Estimate	−0.21	−1.23	−0.19	−0.18	0.01	1.06
	P value	.75	.06	.77	.79	.98	.07
O75223	Gamma-glutamyl cyclotransferase						
	Estimate	−1.20	−2.17	−8.16	−16.07*	−6.96	−13.89*
	P value	.82	.68	.16	7.5E-03	.15	6.2E-03
P13284	Gamma-interferon-inducible lysosomal thiol reductase						
	Estimate	−0.35	−0.63	−0.26	−0.35	0.09	0.27
	P value	.56	.30	.68	.58	.87	.61

(Continued)

TABLE E4. *(Continued)*

Accession no.	Protein name	MRSA lesional vs MSSA lesional sites	MRSA NL vs MSSA NL sites	MRSA lesional vs no *S aureus* lesional sites	MRSA NL vs no *S aureus* NL sites	MSSA lesional vs no *S aureus* lesional sites	MSSA NL vs no *S aureus* NL sites
P09211	Glutathione S-transferase P						
	Estimate	4.99*	2.29	5.21*	2.43	0.22	0.13
	P value	1.3E-03	.11	1.4E-03	.11	.86	.91
P04406	Glyceraldehyde-3-phosphate dehydrogenase						
	Estimate	8.55	8.62	2.06	1.83	−6.50	−6.78
	P value	.13	.13	.73	.76	.20	.18
P00738	Haptoglobin						
	Estimate	4.87	−0.14	10.62	1.36	5.75	1.49
	P value	.36	.98	.07	.81	.23	.75
P04792	Heat shock protein beta-1						
	Estimate	0.90	1.25*	1.35*	1.49*	0.45	0.24
	P value	.15	.05*	.04*	.03*	.41	.66
P69905	Hemoglobin subunit alpha						
	Estimate	−11.57*	−1.92	−3.47	0.46	8.10*	2.38
	P value	1.4E-03	.56	.32	.90	9.6E-03	.42
P68871	Hemoglobin subunit beta						
	Estimate	−18.12*	−1.03	−7.76	1.63	10.36*	2.66
	P value	1.6E-03	.84	.17	.77	.03*	.57
P01876	Ig alpha-1 chain C region						
	Estimate	−2.16	0.31	−6.33*	0.39	−4.17	0.09
	P value	.46	.92	.05*	.90	.11	.97
P01857	Ig gamma-1 chain C region						
	Estimate	−8.76	3.00	−6.12	6.54	2.64	3.54
	P value	.18	.64	.37	.34	.64	.54
P01834	Ig kappa chain C region						
	Estimate	−5.14	4.66	1.09	7.87	6.22	3.22
	P value	.37	.42	.86	.20	.23	.53
P01842	Ig lambda chain C regions						
	Estimate	−14.78*	0.60	−6.92	0.78	7.86	0.18
	P value	9.3E-03	.91	.23	.89	.11	.97
Q9NZH8	Interleukin-1 family member 9						
	Estimate	−0.71	0.49	−0.92	0.21	−0.22	−0.28
	P value	.62	.73	.53	.89	.86	.82
P00338	L-lactate dehydrogenase A chain						
	Estimate	0.07	1.07	0.36	2.53	0.29	1.46
	P value	.97	.62	.87	.27	.88	.45
P31025	Lipocalin-1						
	Estimate	3.55	18.62	3.23	18.04	−0.33	−0.57
	P value	.42	1.7E-04	.48	4.4E-04	.93	.88
Q6S8J3	POTE ankyrin domain family member E						
	Estimate	−1.47	1.02	−0.28	1.55	1.18	0.53
	P value	.37	.54	.87	.37	.42	.71
Q06830	Peroxiredoxin-1						
	Estimate	1.90	2.87	1.81	1.63	−0.09	−1.24
	P value	.29	.11	.34	.39	.95	.44
P32119	Peroxiredoxin-2						
	Estimate	0.99	−0.23	−2.64	−3.55	−3.63	−3.32
	P value	.69	.93	.31	.18	.10	.14
P31151	Protein S100-A7						
	Estimate	11.43	23.11*	3.62	22.77*	−7.81	−0.33
	P value	.16	6.4E-03	.67	.01*	.27	.96
P05109	Protein S100-A8						
	Estimate	5.34*	1.19	3.77	2.84	−1.57	1.65
	P value	.04*	.63	.16	.29	.48	.46
P06702	Protein S100-A9						
	Estimate	1.20	0.04	−0.27	1.93	−1.47	1.89
	P value	.55	.98	.90	.37	.41	.29

(Continued)

TABLE E4. *(Continued)*

Accession no.	Protein name	MRSA lesional vs MSSA lesional sites	MRSA NL vs MSSA NL sites	MRSA lesional vs no *S aureus* lesional sites	MRSA NL vs no *S aureus* NL sites	MSSA lesional vs no *S aureus* lesional sites	MSSA NL vs no *S aureus* NL sites
Q08188	Protein-glutamine gamma-glutamyltransferase E						
	Estimate	1.04	−1.20	−1.55	−0.45	−2.59	0.76
	P value	.80	.77	.72	.92	.48	.84
P14618	Pyruvate kinase isozymes M1/M2						
	Estimate	0.10	1.93	2.30	2.18	2.20	0.25
	P value	.95	.29	.23	.26	.18	.88
Q53RT3	Retroviral-like aspartic protease 1						
	Estimate	−1.29	0.51	−0.02	−0.20	1.27	−0.71
	P value	.33	.70	.99	.88	.28	.55
P02787	Serotransferrin						
	Estimate	−1.51	0.72	12.19	3.37	13.70*	2.65
	P value	.84	.92	.13	.67	.05*	.69
Q96P63	Serpin B12						
	Estimate	−7.70	−12.86	−7.58	−16.72*	0.12	−3.86
	P value	.28	.07	.31	.03*	.98	.54
P29508	Serpin B3						
	Estimate	20.01	6.66	18.46	25.33	−1.54	18.67
	P value	.24	.69	.30	.16	.92	.21
P35237	Serpin B6						
	Estimate	−0.47	−0.54	−0.09	−0.25	0.38	0.29
	P value	.49	.43	.90	.73	.53	.63
P50452	Serpin B8						
	Estimate	−1.14	−4.46	−2.90	−4.83	−1.76	−0.37
	P value	.61	.06	.23	.05	.39	.85
P02768	Serum albumin						
	Estimate	−101.90	4.36	18.34	42.56	120.24*	38.20
	P value	.06	.93	.74	.44	.01*	.41
P00441	Superoxide dismutase [Cu-Zn]						
	Estimate	0.32	1.87	−0.28	1.74	−0.60	−0.13
	P value	.77	.10	.81	.15	.54	.90
P10599	Thioredoxin						
	Estimate	−9.23	−19.96	−11.00	−20.66	−1.77	−0.71
	P value	.51	.16	.46	.17	.89	.96
P19971	Thymidine phosphorylase						
	Estimate	2.27	4.39*	3.33	4.21	1.07	−0.18
	P value	.29	.05*	.15	.07	.57	.92
P60174	Triosephosphate isomerase						
	Estimate	3.40	1.37	1.81	−5.05	−1.58	−6.42
	P value	.67	.86	.83	.55	.83	.37
P25311	Zinc-alpha-2-glycoprotein						
	Estimate	11.34	2.82	−12.50	−8.06	−23.84*	−10.89
	P value	.29	.79	.27	.48	.02*	.26

Comparisons highlighted in gray met the experiment-wise threshold for statistical significance ($P \leq .004$), whereas comparisons with an *asterisk* did not meet the threshold but were of interest for exploratory purposes.
NL, Nonlesional.