MR. RAINER VOEGELI (Orcid ID : 0000-0002-3951-0329)

Article type : Original Article

1) Title of the paper:

The effect of photodamage on the female Caucasian facial stratum corneum corneome using mass spectrometry-based proteomics.

2) Running head:

Corneome of photodamaged stratum corneum.

4) Names of authors:

Rainer Voegeli[1], Jean-Marc Monneuse[2], Rotraut Schoop[1], Beverley Summers[3], Anthony V. Rawlings[4]

5) Names of institutions:

1. DSM Nutritional Products Ltd., Kaiseraugst, Switzerland.
2. Phylogene S.A., Bernis, France.
3. Sefako Makgatho University, Pretoria, South Africa.
4. AVR Consulting Ltd., Northwich, United Kingdom.

6) Corresponding author:

Rainer Voegeli
DSM Nutritional Products Ltd.
P.O. Box 2676, Bldg. 205/315
CH-4002 Basel
Switzerland
Phone: +41 61 815 92 98
Fax: +41 61 815 80 50
Email: rainer.voegeli@dsm.com

7) Funding source:
All funding was provided by DSM Nutritional Products Ltd., Basel, Switzerland.

8) Conflicts of interests and disclosures:
RV and RS employees of DSM. AVR is a consultant to DSM. BS and JMM report no conflicts of interest.

9) Key words:
Skin barrier, photodamaged skin, skin physiology/structure, spectroscopy, stratum corneum, proteomics, corneome.

BACKGROUND: The effect of photodamage on facial stratum corneum (SC) is still poorly understood.

OBJECTIVE: To describe the SC proteome from tape strippings of Caucasian SC from photoexposed cheek and photoprotected post auricular (PA) site a global analysis of photodamage on the skin will be developed leading to a better understanding of keratinocyte signalling pathways and identification of new molecular targets for the treatment of photoaged skin.

METHODS: Female Caucasian subjects had 9 consecutive tape strippings taken from their cheeks and PA site. Proteins were extracted and the trypsin digested peptides were analyzed by nanochromatography coupled to a high-resolution mass spectrometer. Data dependent acquisition allowed protein identification that was processed by Paragon algorithm of Protein Pilot software.

RESULTS: Changes in the levels of epidermal differentiation proteins were apparent indicating poor epidermal differentiation and SC maturation (keratins, cornified envelope (CE) proteins) on photoexposed cheeks. Differences in protease-antiprotease balance were observed for corneodesmolysis (favouring desquamation) and filaggrinolysis (favouring reduced filaggrin processing). 12R-LOX, a CE

maturation enzyme, was reduced in photodamaged skin but not transglutaminases. Changes in signal keratinocyte transduction pathway markers were demonstrated especially by reduced levels of downstream signalling markers such as calreticulin (unfolded protein response; UPR) and increased level of stratifin (target of rapamycin; mTOR). Evidence for impaired proteostasis was apparent by reduced levels of a key proteasomal subunit (subunit beta type-6). Finally, key antioxidant proteins were upregulated except catalase.

CONCLUSION: Clear examples of poor keratinocyte differentiation and associated metabolic and signalling pathways together with reduced SC maturation were identified in photodamaged facial SC. Corneocyte immaturity was evident with changes in CE proteins. Particularly, the reduction in 12R-LOX is a novel finding in photodamaged skin and supports the lack of SC maturation. Moreover, filaggrinolysis was reduced, whereas corneodesmolysis was enhanced. From our results, we propose that there is a poor crosstalk between the keratinocyte endoplasmic reticulum UPR, proteasome network and autophagy machinery that possibly leads to impaired keratinocyte proteostasis. Superimposed on these aberrations is an apparent enhanced mTOR pathway that also contributes to reduced SC formation and maturation. Our results clearly indicate a corneocyte scaffold disorder in photodamaged cheek SC.

Introduction.

Without knowing what consumers want, product development will be meaningless and be like ‘a shot in the dark’. Fortunately, consumer research is continually unravelling the reasoning behind unmet consumer needs. However, without an understanding of the substrate biology behind these unmet consumers’ needs, any

innovation will be like a ‘shot in the dark, while firing blanks’. Thus, to identify new molecular targets for visually dry and rough photodamaged facial skin, a better understanding of the cellular biology and biochemistry is required for the substrate in question. Moreover, without this understanding, predictive *in vitro* models cannot be developed to further probe the relevance of these mechanisms.

Many researchers use surrogate body sites to understand the effects of ingredients on skin, for example, forearm skin, as it is more easily accessible. However, the complexity of facial stratum corneum (SC) barrier and moisturisation has recently been demonstrated and highlights the need for studies to be performed directly on facial skin [1]. As a result this paper is focussed on the effects of photodamage on facial skin, particularly the SC, to better-identify molecular targets for its treatment.

Compared to some other body sites, facial SC has an elevated transepidermal water loss (TEWL), increased corneocyte cohesion but also higher proteolytic activities resulting in premature corneodesmosomal degradation and a thinner tissue [2-4]. This indicates that facial ‘*stratum disjunctum’* is diminished or even no longer present anymore and the reason for the increased SC cohesion is the presence of largely a ‘*stratum compactum’*. The elevated protease activities on the face may be due to a sub-clinical, micro-inflammatory or pre-inflammatory condition induced *e.g.* by environmental effects. Thus, increased protease activities might represent key markers for an underlying and sometimes non-observable skin abnormality. Moreover, differences in lipid and natural moisturizing factor (NMF) levels and composition are known compared with other body sites [5,6]. In particular, reduced NMF levels are apparent on the face presumably due to impaired profilaggrin

expression and its processing, a reduced SC barrier lipid/protein ratio, increased sphingosine/phytosphingosine ceramide levels and reductions in the chain length of ceramides [7-11].

In order to determine the effects of photodamage on skin, we have, like other research groups, compared the differences in the physiology, biochemistry and corneocyte morphology of the SC between samples from photoexposed cheek and photoprotected post-auricular (PA) sites [12]. In Caucasian cheek SC we have found increased plasmin and bleomycin hydrolase, but reduced calpain-1, activities with only marginally increased pyrrolidone carboxylic acid (PCA) levels and greater quantities of immature corneocyte envelopes (CEs) compared with the PA sites. Although this research has contributed to the understanding of the physiology of photoaging of facial SC, more is still to be learned.

Initially the understanding of SC composition has generally been on an analyte-by-analyte basis, by two-dimensional electrophoretic and chromatographic methods together with multiplex enzyme-linked immunosorbent assays. These approaches have been highly successful in helping us to determine the general composition of the SC but each has their limitations. The use of mass spectrometry-based ‘omic’ approaches is on the increase for investigating skin biochemistry especially proteomics [13-19]. Recently these methods have been used to determine differences in the corneome of dry skin on the leg of postmenopausal women and dandruff on the scalp [20-22]. However, these approaches have only recently been used to study the proteome of facial SC.

In order to gain a greater insight into the effects of photodamage on the SC and to identify novel protein markers, label-free mass spectrometry based-proteomics was performed on samples from the cheek and PA of Caucasian women in the current study. Defining differences in the expressed proteins will lead to a new understanding of proteomic changes in the photodamaged epidermis/SC and finally to new molecular targets to mitigate the condition.

Materials and Methods.

Study subjects

The study was a cross-sectional study and was approved by the School of Health Care Sciences Research Committee together with the University Research and Ethics Committee and was conducted in accordance with the Declaration of Helsinki Principles. Written, informed consent was obtained from all participants before enrolment. Six healthy female Caucasian volunteers (39.0 ± 5.3 years old, Fitzpatrick skin phototype II and III), living in Pretoria, South Africa participated in this observational study which took place from the end of November to early December 2013 from a subgroup of the original panel of subjects. The subjects did not apply any dermatological or cosmetic products to their faces for 3 days before expert grading and evaluation of their facial skin. For this 3-day conditioning phase subjects cleansed the face with tepid water in the morning as well as in the evening. Before tape stripping the skin was cleaned by gently swabbing with a cotton pad soaked in distilled water at ambient temperature and allowed to dry for 20 minutes and then acclimatized for 30 minutes at 21 ± 1^{o}C and 35 ± 10% relative humidity [12].

Sample collection and SC protein evaluation.

Nine standard D-Squame® (Cuderm Corporation, Dallas, US) disks with a diameter of 2.2 cm and an area of 3.8 cm^2 were placed on the right cheek (3 cm vertically beneath the outer edge of the eye) and post-auricular area (area opposite to right earlobe). The tapes were applied with 225g cm^{-2} of pressure with a pressure device (Cuderm Corporation, Dallas, US) for 5 seconds and then removed by a single stroke movement. All nine strippings were taken sequentially from each site. In order to minimize variations, the procedure was conducted by the same technician for all volunteers, throughout the study. The interval between the tape strippings was 20 ± 5 seconds [23,24].

Protein extraction, digestion and clean up.

Each tape was placed adhesive side up in its own 20 mL borosilicate scintillation vial (Wheaton; Fisher Scientific, Pittsburgh, Pa). Proteins were extracted using PBS buffer containing 0.2% SDS and 0.5% propylene glycol (Sigma-Aldrich, St. Louis, US) supplemented with 1x HALT protease inhibitors, EDTA-free (Pierce; Thermo Scientific, Rockford, US). The vials were incubated one hour at room temperature in a sonication bath (Sonorex; BANDELIN electronic, Berlin, DE). Proteins were precipitated in 40 mL ice-cold 90% methanol containing of 0.1% formic acid (Sigma-Aldrich, St. Louis, US). Samples were incubated at –20°C overnight, and then centrifuged at 15,000 g at 4°C for 20 minutes. Protein pellets were resuspended in 0.1 M Tris-HCl – 8 M urea buffer (pH 8.5, Euromedex, Souffelweyersheim, FR). The protein content was estimated by BCA assay (Pierce; Thermo Scientific, Rockford, US)**.** The protein extract was digested according to FASP II procedure as described by Wiśniewski *et al.* [25], with slight modifications. The protein extracts (150 µg) were

loaded onto Amicon Ultra 0.5 mL 10 kDa filters (Millipore, Billerica, US), filters were washed with 8 M urea in 0.1 M Tris (pH 8.5) to facilitate removal of protein-bound small molecules. Proteins were reduced with 10 mM dithiothreitol (Euromedex, Souffelweyersheim, FR) for 60 minutes and alkylated with 50 mM iodoacetamide (Sigma-Aldrich, St Louis, US) for 30 minutes. The urea buffer was exchanged by ultrafiltration for ammonium bicarbonate 50 mM (Sigma-Aldrich, St Louis, US). The cleaned proteins were digested overnight on the filter with trypsin at a 1:50 ratio (Promega, Madison, US) and the resulting peptides were released from the filter by centrifugation (14,000g, 20 minutes), followed by a first wash of the filter in 0.5 M NaCl and second wash of the filter by 50 % acetonitrile (both Sigma-Aldrich, St Louis, US). The three fractions were pooled and peptides were diluted at 5% acetonitrile and acidified by 0.1 % trifluoroacetic acid 0.1% final concentration. Peptides were desalted using 500 mg SepPak tC18 sample extraction columns (Waters, Milford, US), eluted with 1.2 mL of 70% acetonitrile, dried and dissolved in 100 µl of water acidified with 0.1% formic acid (Sigma-Aaldrich, St. Louis, US). The peptides amount of each digest was estimated by BCA assay (Pierce; Thermo Scientific, Rockford, US).

Nano-liquid chromatography and tandem mass spectrometry.

Peptide digests (100 ng per run) were loaded onto a nanoACQUITY UPLC Symmetry C18 Trap Column, 180 µm x 20 mm (particle diameter 5 µm, pore size 100 Å) in trap and elute mode with ACQUITY UPLC Peptide BEH C18 nanoACQUITY Column 75 µm x 250 mm (particle diameter 1.7 µm, pore size 130 Å) (Both Waters, Milford, US). The run gradient was performed by Eksigent Ultra Plus nano-LC 2D HPLC (AB Sciex, Framingham, US) system over 90 minutes with a

gradient from 3% to 40% buffer B (buffer A: 0.1% formic acid; buffer B: 95% acetonitrile, 0.1% formic acid) at a flowrate of 300 nl/minute. The Elsigent system was coupled to a TripleTOF® 5600 (AB Sciex, Framingham, US) mass spectrometer interfaced to a nano spray III source. The source parameters were set as follows: IS at 2500 V, Curtain gas at 30 psi, gas sprayer 1 at 1 psi and interface heater temperature at 150°C. The acquisition parameters were as follows: for DDA mode one 250 ms MS scan (>30K resolution). Following each survey MS1 scan, MS/MS spectra for the 30 most abundant parent ions (m/z range 350-1250) were acquired (high sensitivity mode, >15K resolution). For DIA mode one 150 ms MS scan (>30K resolution), followed by 35 fixed SWATH windows each with a 75 ms accumulation time and a 350-1250 m/z range. MS/MS SWATH scans (high sensitivity mode, >15K resolution) were set at 26 amu window, Q1 isolation windows were covering entire mass range [26].

Data analysis and peptide annotation.

DDA spectra processing and database searching was performed with ProteinPilot (v4.5 beta, ABSciex, Framingham, US) using the Paragon algorithm. The search parameters were as follows: sample type: identification; cys alkylation: iodoacetamide; digestion: trypsin; instrument: TripleTOF 5600; special factors: urea denaturation. ID focus: biological modifications. The database was downloaded from Uniprot (June 2014), filtering for reviewed human proteins only (20194 entries). The resulting .group file was loaded into Peakview® (v2.0, AB Sciex, Framingham, US) and peaks from SWATH runs were extracted with a peptide confidence threshold of 99% and a false discovery rate <1%. Label-free quantification was performed by using Marker View (v1.2.1, AB Sciex, Framingham, US). The selection of the proper

peak was performed using the automated assistance of PeakView. The absolute signal of peptide or protein was calculated by summing the extracted area of all unique fragment ions [27]. These annotated fragment ions are referred to as proteins throughout the text of this paper.

Statistical analysis.

For each protein, a linear mixed model was fit to the logtransformed protein measurements with test site as fixed effect and subject pool as random effect followed by an unadjusted post-hoc Tukey test, thus comparing the ethnic groups with each other. Additionally, q-values were calculated (which can be interpreted as adjusted p-values) to take multiplicity issues into account. Each single p-value reports the probability that the observed difference (for this protein and between the two skin sites examined) is due to chance alone. By tradition, a threshold p-value of 0.05 is applied. All comparisons with p-values below that threshold are called “significant” and it is assumed that there is more than noise to it, i.e. that it is a “discovery”. However, since we look at not only one comparison, but a multitude in this analysis (around 1000; 3 group comparisons per protein) a threshold p-value of 0.05 implies in our setting that 5% of all comparisons (i.e. around 50) will be significant due to chance, i.e. will be “false discoveries”. To control the rate of false discoveries (FDR) among all discoveries, we calculated the corresponding q-values, which have also been termed FDR-adjusted p-values. Again, by tradition, a threshold q-value of 0.05 is applied. All comparisons with q-values below that threshold are treated as discoveries, and the theory behind the q-value calculation implies that of these discoveries, only 5% (or x% if another threshold is used) are false discoveries.

Unfortunately, it remains impossible to tell which of the discoveries belong to those 5%. For the resulting p-values of all proteins and comparisons we again calculated q-values.

Results.

A total of 457 SC proteins were identified 436 on the cheek and 253 on the PA site respectively (Supplementary Table I). Comparing between sites a total of 73 were significantly upregulated by factor >2 on the cheek vs the PA site whereas 30 where down regulated by factor <0.5. 232 common proteins were observed on both sites. However, 204 and 21 proteins were found to be different on the cheek and PA sites, respectively. To relate these protein changes to the known phenotypic changes that occur in photodamaged SC the proteins were grouped according to their functions (Table I and Figures 1 and 2). All statistical differences, whether significant p-values, q-values or both, are recorded in Table I.

Serum diffusion linked markers:

32x increased levels of transitional endoplasmic reticulum-adenosine triphosphatase (ATPase) were observed on the cheek which has been related to impaired barrier function. In contrast, 1.7x reduced levels of serum albumin were seen.

Proteases and protease inhibitors:

Nineteen proteases were annotated between the cheek and PA sites. Four were identified to be involved with desquamation (kallikrein 7; 4.27X and 10; 2.39x together with cathepsin L2; 3.96x and D; 1.43X) whereas four are proposed to be involved with filaggrinolysis (retroviral-like aspartic protease 1 (SASPase 1; 5.73X),

calpain-1 (subunits 1; 1.25x and 2; 1.4x), bleomycin hydrolase; 1.73x and caspase 14; 1.66x)). All these were elevated on the cheek. Cathepsin B (1.24x) was slightly elevated and may also be involved in filaggrinolysis.

The levels of serine protease inhibitors serpin B3 (SCCA1; 2.01x), B4 (SCCA2; 1.35x), B5 (3.65x), B7 (13.87x), B8 (1.56x), B12 (1.28x) and B13 (2.72x) levels were increased on the cheek while those of alpha-2 macroglobulin-like 1 (0.67x) and serpin A12 (0.30x) levels were decreased. The cysteine protease inhibitor, cystatin-A, levels (0.85x) were also decreased.

Proteins related to SC cohesion:

Moderate, but statistically significant increases in the levels of desmoglein-1 (1.49x), desmocollin-3 (1.19x) and corneodesmosin (1.20x) were observed on the cheek but the biggest increases were that of epiplakin (3.78x), junction plakoglobin (2.63x) and plakophilin-1 (2.59x). Levels of protein POF1B (1.74x) and desmoplakin (1.61x) were intermediary in level.

Proteins related to corneocyte maturation:

Cornifelin (0.74x), SPRR 1A (0.20x) and 2E (0.22x), loricrin (0.18x), suprabasin (0.43x) and the late envelope protein LEP7/XP32 (0.16x) were significantly lower on the cheek compared with the PA site, whereas SPRR 1B (1.26x) and 2B (1.45x) were increased. Transglutaminases 1 (1.47x) and 3 (1.64x) were elevated, but 12R-LOX levels were decreased (0.31x). A comparison of epidermal lipoxygenase-3 (eLOX3) could not be made as it was only observed on the cheek.

Other enzymes contributing to NMF generation:

Arginase-1 (3.05x), histidine ammonia lyase (2.85x) and gamma-glutamylcyclotransferase (1.10x) were elevated on the cheek. These contribute to urea, urocanic acid (UCA) and PCA respectively.

Differentiation markers: keratins, annexins:

Decreased levels of keratin 1 (0.86x) and 10 (0.79x) were observed on the cheek whereas increased levels of keratins 5 (1.67x) and 14 (1.40x) were found. Levels of keratin 86 (0.77x) and other keratin species were decreased especially keratins 78 (0.12x), 6B (0.25x), 31 (0.25x), 33B (0.36x), 34 (0.34x) 17 (0.46x), 35 (0.48x), 38 (0.59x), 80 (0.59x), 32 (0.69x), whereas 6 other keratin species were increased including keratin 39 (4.68x). Annexins A1 (1.79x), A7 (9.18x) and A11 (5.09x) were found to be decreased on the cheek.

Inflammation markers:

Increased levels of inflammatory proteins were observed on the cheek, especially interleukin-1 receptor antagonist protein (>107x increase). Immunoglobulin C region proteins were increased (alpha-1 chain (17.1x), lambda-3 chain (1.92x), kappa chain (1.59x)) and decreased (gamma-1; 0.19x and -4; 0.7x chains). Secreted Ly-6/uPAR-related protein 1 (5.64x) and Ly6/PLAUR domain-containing protein 3 (4.01x) were also among the most increased of these markers. Interleukin-36 gamma was moderately increased (1.49x).

Membrane trafficking, microtubule and cytoskeleton markers:

Levels of myosin 9 (2.83x) and 14 (2.12x) together with tropomyosin alpha-3 chain (1.19x) were increased, whereas tropomyosin alpha-4 chain (0.52x) and clathrin heavy chain 1 (0.29x) were decreased on the cheek. A variety of cytoskeletal proteins were increased especially gelsolin (>60x).

Proteasome markers:

All proteasome subunit proteins alpha and beta types were upregulated on the cheek except proteasome subunit beta type-6 (0.44x) was down regulated.

Antioxidant markers:

All identified antioxidant markers were upregulated on the cheek except catalase (0.85x). These included glutathione S-transferase P (7.78x), selenium-binding protein 1 (3.85x), glutaredoxin (3.30x), thioredoxin (1.17x), peroxiredoxins 1 (1.15x), 2 (3.14x) and 6 (1.59x), Cu-Zn superoxide dismutase (1.38x) and protein DJ-1 (1.35x).

Heat shock proteins:

Two heat shock proteins were identified to be upregulated on the cheek (70kDa protein 1A/1B; 1.98x and beta-1; 1.23x) and one down regulated on the cheek (HSP 90-alpha, 0.06x).

Signal transduction markers:

Several signal transduction proteins were upregulated on the cheek especially the 14-3-3 proteins (sigma otherwise known as stratifin (19.8x) and zeta/delta (2.36x)).

SC lipid biochemical markers:

SC lipid metabolism enzymes and transport proteins were found to be upregulated on the cheek including phospholipase B-like 1 (4.29x), glucosylcerebrosidase (3.75x), acid ceramidase (1.50x), whereas others were down regulated such as fatty acid binding protein 5 (0.57x) and prosaposin (0.32x).

Antimicrobial peptides:

Dermcidin (0.76x) and lysozyme g-like protein 2 (0.61x) were down regulated on the cheek whereas ribonuclease inhibitor 1 (13.4x) was increased.

Lysosomal markers:

Several lysosomal markers were increased on the cheek indicative of increased lysosomal activity and possibly lamellar granule biogenesis.

Intermediary metabolism enzymes:

Increased levels of glycolytic enzymes were observed on the cheek as well as those involved in amino acid, purine and pyrimidine metabolism, especially malate dehydrogenase (16.3x). Equally, protein synthesis markers were increased on the cheek such as translocon-associated protein subunit alpha (4.43x) and eukaryotic translation initiation factor 6 (3.00x) whereas elongation factor 2 was decreased (0.44x) on the cheek.

Protein folding markers:

Calreticulin levels (0.45x) were decreased on the cheek.

Discussion

We have previously reported on the differences in selected skin physiology measurements, corneocyte morphology and certain SC biochemistry markers between photoexposed cheek and photoprotected PA site [12,28,29]. To understand the relevance of these differences with other proteins in female Caucasian skin, and with the aim of identifying new molecular targets for treating photodamaged skin, we chose to use untargeted label-free mass spectrometry proteomics. Here we discuss the differences in the photodamaged SC corneome to those individual markers that we previously reported.

Previous analysis indicated that reduced keratinocyte differentiation and SC maturation occurred on the photodamaged cheek compared with the PA site and consistent with this we observed decreased keratin 1 and 10 levels in our proteomic approach. Cavusoglu *et al.* also observed decreases in keratins 1, 10 and 17 in subjects with dandruff [21]. In contrast increased levels of the normally basal cell layer expressed keratins 5 and 14 were observed. Also elevated levels of the hyperproliferation marker keratin 16 were also seen consistent with the results of Proksch *et al.* [30]. However, unlike the work of Rice *et al*. [31] on forehead samples, keratin 6A was not observed in the cheek samples and keratin 6B levels were reduced. These and changes in other keratins potentially indicate alterations to the architecture and biomechanical properties of the corneocyte keratin matrix in the photodamaged cheek.

Potentially impaired CE biomechanics were also apparent with the differential expression of certain CE protein markers and associated CE maturation enzymes.

Consistent with this, increased levels of SPRR 1B and 2B while decreased levels of 1A and 2E were found. SPRRs have a specialized role in the CE as they function as cross-bridging agents, which either interconnect or adjoin other CE precursor proteins and affect the biomechanical properties of the CE [31]. However, other CE proteins were also found to be decreased namely cornifelin, suprabasin and especially loricrin together with skin specific protein 32 or LEP7. Loricrin, also, is cross-linked to itself and other SPRR proteins. These are then cross-linked to the periplakin-involucrin-envoplakin scaffold. Subsequently, filaggrin, S100 family proteins and finally the late CE proteins are attached [31]. Although we observed increased levels of involucrin on the cheek we believe this is related to the skin xerosis [30]. The differences in proteome composition in the photodamaged cheek further indicate potential changes to the biomechanical properties of the CE.

Rinnerthaler *et al.* [32] reported some similar changes with intrinsic aging to those that we found in our cheek samples: decreased loricrin and SPRR 1A, 2E, S100A7, A8 and A9 levels. However, unlike the study of Trzeciak *et al*. [33] we observed decreased levels of SPRR1A compared with their recorded increases in subjects with atopic dermatitis. Nevertheless, studying the hand eczema proteome, Molin *et al*. [16] observed some similar changes to those reported here, i.e. increased SPRR2B, but also observed the opposite for other proteins e.g. reduced kallikrein 5 and increased S100A7-9 and 11. Like our study decreased levels of suprabasin were also observed in subjects with dandruff. Any differences in protein levels to our findings probably relate to the differences in (patho)physiology among the different conditions but also possibly differences in extraction methodologies.

Inconsistent with our previous results of reduced CE maturation, levels of peptide sequences for transglutaminases 1 and 3 were found to be increased in the cheek samples [12]. Reduced transglutaminase activities and reduced mass levels are reported in the SC of subjects with post-menopausal dry leg skin, sensitive facial skin conditions,dandruff and xerosis [20,21,34,35]. However, it is possible that the transglutaminases may not be adequately activated or their substrates prepared satisfactorily for their use. Cathepsin D and calpain-1 subunits levels, enzymes that activate transglutaminases, were also found to be elevated on the cheek so these differences do not explain the CE phenotype we previously observed. However, ultraviolet radiation (UVR) leads to increased transglutaminase expression [36,37].

Transglutaminases cross-link CE proteins but they also help to increase the hydrophobicity of the envelope through esterification with ceramides [31]. These ceramides are derived from the very long chain linoleoyl omega-hydroxy ceramides (CER [EOS]) [11]. However, the linoleoyl moiety is first oxidised by two lipoxygenases, 12R-LOX and eLOX3, before being hydrolysed and then the free omega-hydroxy ceramides being utilised by transglutaminase 1 [38]. Levels of eLOX3 were found not to change between the cheek and the PA-samples but the levels of 12R-LOX were dramatically lowered. As a result the attachment of ceramides to the CE by transglutaminase 1 is expected to be decreased and is consistent with the findings of a greater proportion of Nile-red deficient immature CEs previously reported in these samples [12]. This study shows for the first time the relevance of the reduced levels of 12R-LOX in photodamaged SC.

Hirao has demonstrated that the CE's have the potential to mature, increase their hydrophobicity, in the presence of transglutaminase and associated CE protein and lipid components [39]. Nevertheless, immature CE's are also biomechanically weaker [35]. It is also known that CE's are fragile to a sonication stress when there is a loricrin or 12R-LOX deficiency [40,41]. The potential changes in CE biomechanics of the cheek and PA samples are currently under investigation.

Corneodesmolysis is also usually associated with CE maturation and ultimately desquamation [42]. However, in dry skin conditions non-peripheral corneodesmosomal proteins are not effectively proteolysed which also contributes to the immature CE phenotype and a thicker '*stratum compactum'*. Even though the SC is thinner on the face a '*stratum compactum'* phenotype is also apparent indicating the presence of non-peripheral corneodesmosomes [12,43]. Consistent with a lack of CE maturation and changes in desquamation, differences in corneodesmosomal markers were observed between the two body sites. Increased levels of intra-corneocyte corneodesmosomes: epiplakin, junctional plakoglobin, plakophilin-1 and protein POF1B were seen as well as increased levels of CE membrane spanning proteins such as desmoglein 1, corneodesmosin and desmocollin 3. Similarly, Delattre *et al.* [20] also observed increased levels of desmoglein 1, plakoglobin and corneodesmosin whereas surprisingly desmoglein 1 levels were reported to decline in subjects with dandruff. Increased corneodesmosomal markers can be associated with a thickening of the SC but we had already reported a thinning of the SC in the cheek vs the PA sites [12]. As a result there must be increased levels of desquamatory enzymes on the cheek leading to an accelerated shedding of *'stratum disjunctum'* while retaining a *stratum compactum* phenotype.

Indeed, dramatically higher levels of the desquamatory-associated serine protease kallikrein 7 were observed in the cheek samples (4.27x) as well as kallikrein 10 (2.39x) which is consistent with our previously reported enzyme activity data [29]. Moreover, increases in levels of cathepsin L2 and D were observed which have also been associated with desquamation [43]. Caspase-14 levels were higher on the cheek and as this protease are also known to be localised to the corneodesmosome and may be implicated in this process as well. The largest increase in mass levels of potential desquamatory proteases was observed for SASPase (5.73x). However, these activities may or may not be counterbalanced by protease inhibitors [43]. With regard to enhancing kallikrein 7 activity, levels of alpha-2 macroglobulin-like protein 1 and serpin A12 (0.30x) were decreased. Cystatin A (stefin-1) levels were decreased which may enhance cathepsins B, H and L activities. Increased levels of Serpin B13 were also observed on the cheek and may also inhibit cathepsin L, calpain-1 and some serine proteases. Nevertheless, our previous work indicated increased desquamation on the cheek and as a result the protease-antiprotease balance must be in favour of proteolysis on the cheek [12,29]. Increased levels of puromycin-sensitive aminopeptidase may be involved in general proteolysis pathways which was the major protease increased on the cheek samples but its role in corneodesmolysis is unknown. In addition to their effects on controlling endogenous proteases, the protease inhibitors identified may be part of the innate immune system to control microflora proteases [43].

Both Serpin B3 (SCCA1) and serpin B4 (SCCA2) were found to be increased in barrier compromised conditions [44,45]. Serpin B3 is known to inhibit cysteine proteases such as cathepsin L2 whereas serpin B4 inhibits serine and cysteine

proteases but increases in their levels may be a reflection of SC barrier abrogation [46,47]. Katagiri *et al.* [45] showed that upregulation of serpin B3 is associated with barrier disruption in psoriasis and following UV irradiation. Yamane *et al.* [44] also found increased serpin B4 levels in the SC of atopic patients. Moreover, Sivaprasad *et al.* [48] also reported that increased TEWL was attenuated by an absence of serpin B3. Broccado *et al.* [14] also found increased serpin B3 in atopic subjects. Overexpression of serpin B3 also promotes epithelial proliferation [14]. In keratinocytes Serpin B3 binds to c-Jun N-terminal kinase and upon its activation is translocated to the nucleus to play a role in UV-induced apoptotic cell death. In parakeratotic corneocytes it can be shown to be colocalised with undigested nuclei and it may cause poor barrier function by disrupting the internal structure of the corneocytes [45].

Although facial SC contains significantly lower levels of NMF compared with other body sites we have shown that there are no differences in PCA levels between the cheek and PA sites [5,6,29]. However, UV irradiation is known to increase the expression of filaggrin and transglutaminase in mouse skin [36,37]. Despite this finding, filaggrin (and transglutaminase 1 together with desmoplakin-1 and -2) levels are reported to be lower at the bottom of a wrinkle in human skin [8]. In contrast to this latter finding we also observed increased levels of filaggrin with slight increases in the levels of filaggrin-2 in the cheek compared with the PA site. Thus, filaggrinolysis appears to be less effective on the cheek compared with the PA site. Despite this, increased levels of SASPase, bleomycin hydrolase, caspase-14 and calpain-1 were observed on the cheek. The latter is not consistent with our activity measurements and its activity may be compensated by increased expression of

calpastatin on the cheek (see Supplementary Table I) [29]. Equally, the increased levels of prostasin on the cheek should also increase the processing of profilaggrin [43]. The slight increased cathepsin B levels should also contribute to filaggrinolysis and be further enhanced by the slightly lowered levels of cystatin-A [43,50]. Likewise, Kawada *et al*. [51] also reported that cathepsin L degrades filaggrin but serpin B13 (Hurpin) as does serpin B3 inhibits it, which may be mitigating filaggrinolysis [52]. Furin, a member of the subtilisin/kexin-like mammalian proprotein convertases is also involved the (pro)filaggrin processing and its inhibitor serpin B8 is increased in the cheek samples which may account for the lack of total (pro)filaggrin processing on the cheek compared with the PA site [43,53]. The UCA and PCA generating enzymes, histidine ammonia lyase (3x) and gamma-glutamyl cyclotransferase respectively, were also increased together with arginase-1 (3x), that contributes to urea formation [54]. Thus, the protease-antiprotease balance maybe reducing (pro)filaggrin processing being in favour of the antiproteases.

Pro-cathepsin H was observed on the cheek samples (Supplementary table I). We cannot comment on the relative differences of this inhibitor to the PA site as it was not detected in this site. However, as has been shown by Naeem *et al.* [55] reduced cathepsin H activities have been observed in conditions where increased mTOR activity (increased regulatory associated protein of mTOR / raptor) was observed, e.g. atopic dermatitis resulting in reduced filaggrin processing. *Choi et al*. [56] have also reported increased mTOR complex 2/ raptor independent companion of mTOR expression following UVR which leads to associated nuclear factor kappa-light-chain-enhancer of activated B cells/ NF-κB activation and thereby may reduce

filaggrin expression. Increased mTOR signalling pathways following UV irradiation has also been documented [57].

Although mTORC1 and mTORC2 regulate epidermal barrier formation their over stimulation appears to be a problem for filaggrinolysis [58]. Although we did not detect specific mTOR-associated proteins, increased mTOR activity results in reduced autophagy, a process known to be essential for keratinocyte differentiation [59,60]. However, certain proteins are increased on the cheek indicative of enhanced mTOR activity e.g. increased levels of the adapter signalling protein, stratifin (14-3-3σ) were observed on the cheek [61]. Raaby *et al.* [62] reported increased stratifin expression in psoriasis and contact dermatitis and its levels are also reported to be increased following UV irradiation leading to decreased profilaggrin expression [61]. Thus, increased mTOR activity is likely in the cheek samples.

Increased filaggrinolysis is also known to occur at the depth in the SC where increased transglutaminase activity occurs [42]. This coincides with changes in the CE phenotype. Presumably increased 12R-LOX activities occur at the same depth. Thus, the reduced 12R-LOX levels we observed on the cheek may also contribute to a reduced profilaggrin processing. Indeed, this has been shown in 12R-LOX knockout mouse models [63].

S100 proteins are small calcium binding proteins but their roles in skin are largely unknown [64]. Increased levels of S100A16 were observed in the cheek which is normally just found in spinous layers that may be indicative of poor differentiation. Equally, reduced levels of S100A3, A7, A8, A9 and A14 were seen. S100A7 is

normally associated with the SC whereas S100A8, A9 and A14 are normally spinous or granular layer proteins. In contrast, increased levels of S100A8 and A9 were observed in subjects with dandruff and psoriasis [64]. The heterodynamic complex of S100A8 and A9 (calprotectin) functions as antimicrobial peptide and as we observed decreased levels this may indicate that the cheek might be more susceptible to antimicrobial attack. Likewise, reduced levels of other antimicrobial peptides such as dermcidin and lysozyme g-like protein 2 might contribute to the reduced innate immunity [43]. Similarly, reduced cystatin A will reduce innate immunity whereas increases in other serpins will be beneficial. Increases in RNA inhibitor (RNH1) may also prevent RNAs from functioning [65]. The role of S100A3 and A14 is unknown. S100A7 which also binds epidermal fatty acid protein (eFABP) suggests it has a role in epidermal lipid barrier formation and its reduced levels in the cheek also indicate reduced keratinocyte differentiation potential [66].

Epidermal fatty acid binding protein, E-FABP/FABP5 is actively overexpressed in hyperproliferative diseases such as psoriasis [67]. However, in keratinocytes lacking FABP5 decreased expression of loricrin, involucrin and keratin 1 was observed [68]. This calycin delivers fatty acids intracellularly to peroxisome proliferator receptors [69]. We observed reduced FABP5 levels which may be contributing to the poor differentiation status of the UV exposed cheek SC.

Similar to results of subjects with dandruff we observed increased calmodulin-like proteins 3 and 5 on photodamaged cheek [21]. These changes have also been observed in subjects with atopic dermatitis and psoriasis [70,71]. Calmodulin-like proteins interact with stratifin and control some of the late differentiation genes [72].

These proteins may be increased to try and improve keratinocyte differentiation but clearly this is not occurring on the cheek. These proteins also upregulate myosins required for cell motility such as myosin-10 [73]. We observed increases in myosin-9 and -14 possibly being related to a hyperproliferative condition.

Although there is redundancy in their function we observed increases in the levels of annexin 1, 7 and 11 indicating reduced keratinocyte differentiation potential [74]. Annexin A2 is increased in dry skin but together with annexin A3 it was reported to be decreased in dandruff, whereas A1 was increased [20,21]. Annexin 7 is reported to mobilize calcium from inositol-1,4,5-triphosphate-sensitive endoplasmic reticulum stores [75].

Gelsolin, an actin binding protein, alpha actinin and filamin are usually less abundant in suprabasal cells compared with basal keratinocytes [76]. Thus, our finding of greatly increased levels of gelsolin on the cheek indicated a hyperproliferative epidermis. Interestingly, increased plasma gelsolin levels are known in atopic dermatitis [77]. This hyperproliferation is also indicated by the increases levels of glycolytic enzymes on the cheek. Broccardo *et al*. [14] also found increased α-enolase and triosephosphate isomerase in atopic dermatitis indicative of increased epidermal hyperproliferation.

Secreted Ly-6/uPAR-related protein 1 is a selective antagonist of the nicotinic acetylcholine receptor that reduces keratinocyte proliferation [78]. However, it is also reported to be pro-apoptotic increasing caspase 3 and transglutaminase 3 levels [79]. We observed increased levels so the skin may be trying to mitigate the

excessive proliferation on the cheek and could account for the increases in levels of transglutaminase 3 we observed. However, transglutaminases may be increased to protect against photodamage [80].

Clearly, the cheek is undergoing UV-induced oxidative stress. Although IL-1 receptor antagonist protein can be associated with keratinocyte differentiation we believe the increased levels together with increases in other inflammatory proteins were indicative of UV-induced inflammation on the cheek [81, 82].

To counteract this, the skin will attempt to mitigate any inflammation and upregulate its antioxidant systems and we observed increases in many antioxidant proteins on the cheek. However, we observed reduced catalase levels. Similar to our cheek samples, Ryu *et al*. [83] reported increased expression of glutathione S transferase 1 and peroxiredoxin 2 in psoriatic skin as was also shown for dandruff SC. Declercq *et al*. [84] have previously reported decreased catalase in the SC in the summer months of the year clearly as a result of UV stress similar to our samples. Protein deglycase DJ-1 was also increased on the cheek which is known to be elevated in UV-induced skin damage [85]. Increased levels of malate dehydrogenase were also observed on the cheek which reportedly increases the levels of the cellular antioxidant reduced nicotinamide adenine dinucleotide while gamma-glutamyl hydrolase levels were also increased that is involved in folate transport [86,87]. An increased level of thioredoxin was also observed presumably to control the redox state of the cells [88]. HSP27 is increased in dry skin but we observed HSP90α to be decreased in our samples indicating that there may be reduced cell mobility [89]. HSP701A levels are higher in darker skin phenotypes and our increases may be

related to an increased tanning response on the cheeks [90]. Overall the skin is trying to defend itself against further oxidative stress. mTOR activity may also be influencing the expression of these proteins as Song *et al.* [91] have shown that autophagy inhibition by using keratinocytes with inactivated autophagy related 7 gene underwent more oxidative stress to an external challenge. Thus, over activation of the mTOR pathway, which inhibits autophagy, will likely have the same effects and may result in some of the changes we have observed in other proteins.

Calreticulin levels were decreased on the cheek. Calreticulin is an intracellular chaperone that is critical for the proper folding and transport of proteins through the endoplasmic reticulum and recruits cells during wound healing [92]. The lack of this chaperone leads to increased endoplasmic reticulum (ER) stress which can activate the unfolded protein response (UPR). However, ER signalling leading to an elevated UPR in response to UVB has been reported but clearly increased levels of unfolded proteins can over activate this mechanism [93]. A mild UPR has been reported to increase the innate immunity β-defensin antimicrobial peptides through terminal differentiation [94]. However, in the cheek samples we observed decreased levels of antimicrobial peptides and increased ribonuclease inhibitor levels which again indicate poor keratinocyte differentiation possibly due to a deficiency in the UPR.

Usually, proteasomes degrade unfolded proteins. However, we observed a proteasome insufficiency in the cheek samples because of reduced levels of one of the essential 20S proteasome subunits, namely PSMB6 [95]. Impairment of proteasome function on UV-irradiation of keratinocytes has been reported [96]. Also proteasome inhibitor treatments lead to reductions in calreticulin [97]. The likely

impaired proteasome functioning in the cheek samples may be the cause of the reduced calreticulin and thereby reduced UPR.

Our analysis of cheek samples indicates poor crosstalk between the keratinocyte ER UPR-proteasome network-autophagy mechanisms and mTOR. UV irradiation clearly leads to oxidation of cellular proteins and as a result control of proteostasis is important. Keratinocytes in particular need to control protein overload in the ER otherwise this will lead to ER stress due to hydrophobic residues exposed from unfolded or misfolded proteins interacting with cellular membranes. This normally leads to an UPR to mitigate these problems and damaged proteins are translocated to the cytosol to be degraded by proteasomes. Autophagy is another route to disposing of damaged proteins as well as organelle degradation. mTOR is also important in these processes as it negatively and positively influences autophagy and the proteasome mechanisms. Nevertheless, these processes are important for terminal differentiation. However, when these mechanisms are under- or over-activated as appears on female Caucasian cheeks, disturbed or poor keratinocyte differentiation events occur [98]. The UPR process can be reduced in diseases such as psoriasis which may lead to reduced filaggrin/NMF levels, whereas it can be over activated in disorders such as keratosis linearis with ichthyosis congenital and keratoderma syndrome (KLICK) that results from a deficiency of the proteasome maturation protein (POMP) leading to proteasome insufficiency which in turn leads to a problem with profilaggrin processing and the later differentiation events [99]. Activation of mTOR also occurs in many dermatological disorders and the resulting blockage of autophagy leads to poor differentiation events [100]. The UPR response is also believed to activate autophagy but in our clinical samples poor differentiation

was still apparent and as a result the mTOR response may be over riding any UPR signalling [101]. Thus, the UPR and proteasome mechanisms appear to be reduced on the cheek whereas mTOR activity is increased.

It should be borne in mind, however, that although a tremendous amount of information has been gathered on the SC corneome, proteomics has its limitations. In this study we have not evaluate post-translational processing protein events and this approach does not provide information on the activity and location of the proteins identified. New target proteins need to be validated with activity and localisation measurements.

In conclusion, we have observed a complex SC corneome in photodamaged skin of the face that shows some differences and similarities to that reported for dandruff, dry skin and skin diseases (atopic dermatitis, psoriasis). These similarities or differences may relate to the underlying diversity in the (patho)physiology of these disparate conditions and/or differences in methodology or selection of body site. Nevertheless, this study broadens the catalogue of the proteins found within the SC and especially in photodamaged sites. Relating to our previously-published findings on SC of cheek compared with that of the PA site, clear differences in corneocyte and CE protein components together with CE maturations enzymes were observed and diametrically opposed changes in the protease-antiprotease balance were measured for corneodesmolysis and filaggrinolysis. From these findings, we expect proteolysis to be favoured for corneodesmolysis causing premature desquamation on the face but protease inhibition for filaggrinolysis on the cheek compared with the PA site. Moreover, 12R-LOX, an upstream CE maturation enzyme was identified as

a key new target for photodamaged skin as its levels were lowered. This enzyme would normally provide the substrate for transglutaminases to hydrophobe the CE. The levels of transglutaminases were unchanged in this study. These enzyme and structural CE protein changes indicate a corneocyte scaffold disorder in photodamaged facial skin. Furthermore, this study provides insights into the potential molecular mechanisms involved in SC maturation/keratinocyte differentiation and their aberration in photodamaged skin. In this respect, analysis of proteins indicated a perturbed cross talk of keratinocyte ER UPR-proteasome network-autophagy mechanisms with an enhancement of mTOR activity in keratinocytes. Changes in the levels and activities of these proteins needs further validation as only peptide fragments have been annotated. Nevertheless, the biochemical diversity of the cheek and PA SC corneome identified in this study has highlighted new molecular pathways/targets that need to be influenced to better treat photodamaged skin.

Legends.

Table I. Comparison of proteins between stratum corneum samples of photodamaged cheek and photoprotected post-auricular sites in Caucasian women.

Figure 1. Schematic of relevant stratum corneum maturation protein changes between stratum corneum samples of photodamaged cheek and photoprotected post-auricular sites in photodamaged Caucasian women.

Figure 2. Comparison of fold increase of proteins, grouped in protein families, of stratum corneum samples on photodamaged cheek versus photoprotected post-auricular sites in Caucasian women. Values >log 1 correspond to an increased and values <log 1 to a decreased protein level on cheek.

Series	Protein family
1	Proteases
2	Protease inhibitors
3	Corneodesmosome consistuents
4	Cornified envelope consitituents
5	Cornified envelope processing enzymes
6	Filaggrin/NMF and its processing enzymes
7	Keratins
8	Calcium binding proteins / AMP
9	Glycoprotein folding protein
10	Annexins
11	Inflammation-associated proteins
12	Microtubules / tubulins
13	Membrane trafficking / myosin
14	Actin binding proteins / cytoskeleton
15	Antimicrobials
16	Proteasome
17	Protein folding control
18	Antioxidant related proteins
19	Heat shock proteins
20	Signal transduction proteins
21	SC lipid metabolism / transport proteins / enzymes
22	Nucleotide metabolism
23	Secreted proteins
24	Lysosomal proteins
25	Glycolysis / Krebs cycle enzymes
26	Serum diffusion markers
27	Protein synthesis
28	Aminoacid /purine / pyrimidine metabolism
29	Retinoid metabolism

Supplementary Table I. Documentation of all proteins observed between stratum corneum samples of photodamaged cheek and photoprotected post-auricular sites in Caucasian women.

Acknowledgements.

This study was financially supported by DSM Nutritional Products Ltd., Basel, Switzerland. We would like to thank Isabelle Metton and Gilbert Skorski of Phylogene, Bernis, France, and Angelika Friedel and Igor Bendik of DSM Nutritional Products Ltd., Basel, Switzerland for their consulting and support in proteomics but also Lebogang Kgatuke, Marlize Lategan, Caroline Moeletsi and Lee-Ann Raaff of the Photobiology Laboratory, Medunsa Campus, Sefako Makgatho University, Pretoria, South Africa, for their enthusiasm in conducting the clinical aspect of the study. Most importantly, we would like to thank the volunteers for their willing and valuable participation in this study.

References.

1. Voegeli, R., Rawlings, A.V., Seroul, P. and Summers, B. A novel continuous colour mapping approach for visualization of facial skin hydration and transepidermal water loss for four ethnic groups. *Int J Cosmet Sci.* 37(6):595-605 (2015).

2. Kobayashi, H. and Tagami, H. Distinct locational differences observable in biophysical functions of the facial skin: with special emphasis on the poor functional properties of the stratum corneum of the perioral region. *Int J Cosmet Sci.* 26(2):91-101 (2004).

3. Voegeli, R., Rawlings, A.V., Doppler, S., Heiland, J. and Schreier, T. Profiling of serine protease activities in human stratum corneum and detection of a stratum corneum tryptase-like enzyme. *Int J Cosmet Sci.* 29(3):191-200 (2007).

4. Mohammed, D., Matts, P.J., Hadgraft, J. and Lane, M.E. Variation of stratum corneum biophysical and molecular properties with anatomic site. *AAPS J.* 14(4):806-12 (2012).

5. Koyama, J., Horii, I., Kawasaki, K., Nakayama, Y., Morikawa, Y., Mitsui, T., et al. Free amino acids of stratum corneum as a biochemical marker to evaluate dry skin. *J Soc Cosmet Chem.* 35:183-95 (1984).

6. Yang, Q. and Guy, R.H. Characterisation of skin barrier function using bioengineering and biophysical techniques. *Pharm Res.* 32(2):445-57 (2015).

7. Tezuka, T., Qing, J., Saheki, M., Kusuda, S. and Takahashi, M. Terminal differentiation of facial epidermis of the aged: immunohistochemical studies. *Dermatology.* 188(1):21-4 (1994).

8. Contet-Audonneau, J.L., Jeanmaire, C. and Pauly, G. A histological study of human wrinkle structures: comparison between sun-exposed areas of the face, with or without wrinkles, and sun-protected areas. *Br J Dermatol.* 140(6):1038-47 (1999).

9. Masukawa, Y., Narita, H., Sato, H., Naoe, A., Kondo, N., Sugai, Y., et al. Comprehensive quantification of ceramide species in human stratum corneum. *J Lipid Res.* 50(8):1708-19 (2009).

10. Ishikawa, J., Shimotoyodome, Y., Ito, S., Miyauchi, Y., Fujimura, T., Kitahara, T., et al. Variations in the ceramide profile in different seasons and regions of the body contribute to stratum corneum functions. *Arch Dermatol Res.* 305(2):151-62 (2013).

11. Moore, D.J. and Rawlings, A.V. The chemistry, function and (patho)physiology of stratum corneum barrier ceramides. *Int J Cosmet Sci.* (2017).

12. Voegeli, R., Rawlings, A.V. and Summers, B. Facial skin pigmentation is not related to stratum corneum cohesion, basal transepidermal water loss, barrier integrity and barrier repair. *Int J Cosmet Sci.* 37(2):241-52 (2015).

13. Raymond, A.A., Gonzalez de Peredo, A., Stella, A., Ishida-Yamamoto, A., Bouyssie, D., Serre, G., et al. Lamellar bodies of human epidermis: proteomics characterization by high throughput mass spectrometry and possible involvement of CLIP-170 in their trafficking/secretion. *Mol Cell Proteomics.* 7(11):2151-75 (2008).

14. Broccardo, C.J., Mahaffey, S.B., Strand, M., Reisdorph, N.A. and Leung, D.Y. Peeling off the layers: skin taping and a novel proteomics approach to study atopic dermatitis. *J Allergy Clin Immunol.* 124(5):1113-5 e1-11 (2009).

15. Sakabe, J., Kamiya, K., Yamaguchi, H., Ikeya, S., Suzuki, T., Aoshima, M., et al. Proteome analysis of stratum corneum from atopic dermatitis patients by hybrid quadrupole-orbitrap mass spectrometer. *J Allergy Clin Immunol.* 134(4):957-60 e8 (2014).

16. Molin, S., Merl, J., Dietrich, K.A., Regauer, M., Flaig, M., Letule, V., et al. The hand eczema proteome: imbalance of epidermal barrier proteins. *Br J Dermatol.* 172(4):994-1001 (2015).

17. Lundberg, K.C., Fritz, Y., Johnston, A., Foster, A.M., Baliwag, J., Gudjonsson, J.E., et al. Proteomics of skin proteins in psoriasis: from discovery and verification in a mouse model to confirmation in humans. *Mol Cell Proteomics.* 14(1):109-19 (2015).

18. Winget, J.M., Finlay, D., Mills, K.J., Huggins, T., Bascom, C., Isfort, R.J., et al. Quantitative Proteomic Analysis of Stratum Corneum Dysfunction in Adult Chronic Atopic Dermatitis. *J Invest Dermatol.* 136(8):1732-5 (2016).

19. Mehul, B., Laffet, G., Seraidaris, A., Russo, L., Fogel, P., Carlavan, I., et al. Noninvasive proteome analysis of psoriatic stratum corneum reflects pathophysiological pathways and is useful for drug profiling. *Br J Dermatol.* (2017).

20. Delattre, C., Winstall, E., Lessard, C., Donovan, M., Simonetti, L., Minondo, A.M., et al. Proteomic analysis identifies new biomarkers for postmenopausal and dry skin. *Exp Dermatol.* 21(3):205-10 (2012).

21. Rice, R.H., Bradshaw, K.M., Durbin-Johnson, B.P., Rocke, D.M., Eigenheer, R.A., Phinney, B.S., et al. Distinguishing ichthyoses by protein profiling. *PLoS One.* 8(10):e75355 (2013).

22. Cavusoglu, N., Delattre, C., Donovan, M., Bourassa, S., Droit, A., El Rawadi, C., et al. iTRAQ-based quantitative proteomics of stratum corneum of dandruff scalp reveals new insights into its aetiology and similarities with atopic dermatitis. *Arch Dermatol Res.* 308(9):631-42 (2016).

23. Loffler, H., Dreher, F. and Maibach, H.I. Stratum corneum adhesive tape stripping: influence of anatomical site, application pressure, duration and removal. *Br J Dermatol.* 151(4):746-52 (2004).

24. Breternitz, M., Flach, M., Prassler, J., Elsner, P. and Fluhr, J.W. Acute barrier disruption by adhesive tapes is influenced by pressure, time and anatomical location: integrity and cohesion assessed by sequential tape stripping. A randomized, controlled study. *Br J Dermatol.* 156(2):231-40 (2007).

25. Wisniewski, J.R., Zougman, A., Nagaraj, N. and Mann, M. Universal sample preparation method for proteome analysis. *Nat Methods.* 6(5):359-62 (2009).

26. Gillet, L.C., Navarro, P., Tate, S., Rost, H., Selevsek, N., Reiter, L., et al. Targeted data extraction of the MS/MS spectra generated by data-independent

acquisition: a new concept for consistent and accurate proteome analysis. *Mol Cell Proteomics.* 11(6):O111 016717 (2012).

27. Shilov, I.V., Seymour, S.L., Patel, A.A., Loboda, A., Tang, W.H., Keating, S.P., et al. The Paragon Algorithm, a next generation search engine that uses sequence temperature values and feature probabilities to identify peptides from tandem mass spectra. *Mol Cell Proteomics.* 6(9):1638-55 (2007).

28. Raj, N., Voegeli, R., Rawlings, A.V., Gibbons, S., Munday, M.R., Summers, B., et al. Variation in stratum corneum protein content as a function of anatomical site and ethnic group. *Int J Cosmet Sci.* 38(3):224-31 (2016).

29. Raj, N., Voegeli, R., Rawlings, A.V., Summers, B., Munday, M.R. and Lane, M.E. Variation in the activities of late stage filaggrin processing enzymes, calpain-1 and bleomycin hydrolase, together with pyrrolidone carboxylic acid levels, corneocyte phenotypes and plasmin activities in non-sun-exposed and sun-exposed facial stratum corneum of different ethnicities. *Int J Cosmet Sci.* 38(6):567-75 (2016).

30. Engelke, M., Jensen, J.M., Ekanayake-Mudiyanselage, S. and Proksch, E. Effects of xerosis and ageing on epidermal proliferation and differentiation. *Br J Dermatol.* 137(2):219-25 (1997).

31. Candi, E., Schmidt, R. and Melino, G. The cornified envelope: a model of cell death in the skin. *Nat Rev Mol Cell Biol.* 6(4):328-40 (2005).

32. Rinnerthaler, M., Duschl, J., Steinbacher, P., Salzmann, M., Bischof, J., Schuller, M., et al. Age-related changes in the composition of the cornified envelope in human skin. *Exp Dermatol.* 22(5):329-35 (2013).

33. Trzeciak, M., Sakowicz-Burkiewicz, M., Wesserling, M., Dobaczewska, D., Glen, J., Nowicki, R., et al. Expression of Cornified Envelope Proteins in Skin and Its

Relationship with Atopic Dermatitis Phenotype. *Acta Derm Venereol.* 97(1):36-41 (2017).

34. Raj, N., Voegeli, R., Rawlings, A.V., Doppler, S., Imfeld, D., Munday, M.R., et al. A fundamental investigation into aspects of the physiology and biochemistry of the stratum corneum in subjects with sensitive skin. *Int J Cosmet Sci.* 39(1):2-10 (2017).

35. Harding, C.R., Long, S., Richardson, J., Rogers, J., Zhang, Z., Bush, A., et al. The cornified cell envelope: an important marker of stratum corneum maturation in healthy and dry skin. *Int J Cosmet Sci.* 25(4):157-67 (2003).

36. Lee, D.S., Quan, G., Choi, J.Y., Kim, S.Y. and Lee, S.C. Chronic ultraviolet radiation modulates epidermal differentiation as it up-regulates transglutaminase 1 and its substrates. *Photodermatol Photoimmunol Photomed.* 21(1):45-52 (2005).

37. Terazawa, S., Mori, S., Nakajima, H., Yasuda, M. and Imokawa, G. The UVB-Stimulated Expression of Transglutaminase 1 Is Mediated Predominantly via the NFkappaB Signaling Pathway: New Evidence of Its Significant Attenuation through the Specific Interruption of the p38/MSK1/NFkappaBp65 Ser276 Axis. *PLoS One.* 10(8):e0136311 (2015).

38. Munoz-Garcia, A., Thomas, C.P., Keeney, D.S., Zheng, Y. and Brash, A.R. The importance of the lipoxygenase-hepoxilin pathway in the mammalian epidermal barrier. *Biochim Biophys Acta.* 1841(3):401-8 (2014).

39. Hirao, T. Involvement of transglutaminase in ex vivo maturation of cornified envelopes in the stratum corneum. *Int J Cosmet Sci.*25(5),245-57 (2003)

40. Koch, P.J., de Viragh, P.A., Scharer, E., et al. Lessons from loricrin-deficient mice: compensatory mechanisms maintaining skin barrier function in the absence of a major cornified envelope protein. *J Cell Biol*.151(2),389-400 (2000).

41. Epp, N., Fürstenberger, G., Mülle,r K. et al. 12R-lipoxygenase deficiency disrupts epidermal barrier function. *J Cell Biol*. 9;177(1), 173-82 (2007)]

42. Rawlings, A.V. Molecular basis for stratum corneum maturation and moisturization. *Br J Dermatol.* 171 Suppl 3:19-28 (2014).

43. Rawlings, A.V. and Voegeli, R. Stratum corneum proteases and dry skin conditions. *Cell Tissue Res.* 351(2):217-35 (2013).

4. Yamane, Y., Moriyama, K., Yasuda, C., Miyata, S., Aihara, M., Ikezawa, Z., et al. New horny layer marker proteins for evaluating skin condition in atopic dermatitis. *Int Arch Allergy Immunol.* 150(1):89-101 (2009).

45. Katagiri, C., Iida, T., Nakanishi, J., Ozawa, M., Aiba, S. and Hibino, T. Up-regulation of serpin SCCA1 is associated with epidermal barrier disruption. *J Dermatol Sci.* 57(2):95-101 (2010).

46. Schick, C., Kamachi, Y., Bartuski, A.J., Cataltepe, S., Schechter, N.M., Pemberton, P.A., et al. Squamous cell carcinoma antigen 2 is a novel serpin that inhibits the chymotrypsin-like proteinases cathepsin G and mast cell chymase. *J Biol Chem.* 272(3):1849-55 (1997).

47. Schick, C., Pemberton, P.A., Shi, G.P., Kamachi, Y., Cataltepe, S., Bartuski, A.J., et al. Cross-class inhibition of the cysteine proteinases cathepsins K, L, and S by the serpin squamous cell carcinoma antigen 1: a kinetic analysis. *Biochemistry.* 37(15):5258-66 (1998).

48. Sivaprasad, U., Kinker, K.G., Ericksen, M.B., Lindsey, M., Gibson, A.M., Bass, S.A., et al. SERPINB3/B4 contributes to early inflammation and barrier dysfunction in an experimental murine model of atopic dermatitis. *J Invest Dermatol.* 135(1):160-9 (2015).

49. Lunardi, F., Villano, G., Perissinotto, E., Agostini, C., Rea, F., Gnoato, M., et al. Overexpression of SERPIN B3 promotes epithelial proliferation and lung fibrosis in mice. *Lab Invest.* 91(6):945-54 (2011).

50. Kawada, A., Hara, K., Morimoto, K., Hiruma, M. and Ishibashi, A. Rat epidermal cathepsin B: purification and characterization of proteolytic properties toward filaggrin and synthetic substrates. *Int J Biochem Cell Biol.* 27(2):175-83 (1995).

51. Kawada, A., Hara, K., Hiruma, M., Noguchi, H. and Ishibashi, A. Rat epidermal cathepsin L-like proteinase: purification and some hydrolytic properties toward filaggrin and synthetic substrates. *J Biochem.* 118(2):332-7 (1995).

52. Welss, T., Sun, J., Irving, J.A., Blum, R., Smith, A.I., Whisstock, J.C., et al. Hurpin is a selective inhibitor of lysosomal cathepsin L and protects keratinocytes from ultraviolet-induced apoptosis. *Biochemistry.* 42(24):7381-9 (2003).

53. Izaguirre, G., Qi, L., Lima, M. and Olson, S.T. Identification of serpin determinants of specificity and selectivity for furin inhibition through studies of alpha1PDX (alpha1-protease inhibitor Portland)-serpin B8 and furin active-site loop chimeras. *J Biol Chem.* 288(30):21802-14 (2013).

54. Harding, C.R., Aho, S. and Bosko, C.A. Filaggrin - revisited. *Int J Cosmet Sci.* 35(5):412-23 (2013).

55. Naeem, A.S., Tommasi, C., Cole, C., Brown, S.J., Zhu, Y., Way, B., et al. A mechanistic target of rapamycin complex 1/2 (mTORC1)/V-Akt murine thymoma viral oncogene homolog 1 (AKT1)/cathepsin H axis controls filaggrin expression and processing in skin, a novel mechanism for skin barrier disruption in patients with atopic dermatitis. *J Allergy Clin Immunol.* 139(4):1228-41 (2017).

56. Choi, Y.J., Moon, K.M., Chung, K.W., Jeong, J.W., Park, D., Kim, D.H., et al. The underlying mechanism of proinflammatory NF-kappaB activation by the mTORC2/Akt/IKKalpha pathway during skin aging. *Oncotarget.* 7(33):52685-94 (2016).

57. Bermudez, Y., Stratton, S.P., Curiel-Lewandrowski, C., Warneke, J., Hu, C., Bowden, G.T., et al. Activation of the PI3K/Akt/mTOR and MAPK Signaling Pathways in Response to Acute Solar-Simulated Light Exposure of Human Skin. *Cancer Prev Res (Phila).* 8(8):720-8 (2015).

58. Ding, X., Bloch, W., Iden, S., Ruegg, M.A., Hall, M.N., Leptin, M., et al. mTORC1 and mTORC2 regulate skin morphogenesis and epidermal barrier formation. *Nat Commun.* 7:13226 (2016).

59. Strozyk, E. and Kulms, D. The role of AKT/mTOR pathway in stress response to UV-irradiation: implication in skin carcinogenesis by regulation of apoptosis, autophagy and senescence. *Int J Mol Sci.* 14(8):15260-85 (2013).

60. Aymard, E., Barruche, V., Naves, T., Bordes, S., Closs, B., Verdier, M., et al. Autophagy in human keratinocytes: an early step of the differentiation? *Exp Dermatol.* 20(3):263-8 (2011).

61. Adachi, H., Murakami, Y., Tanaka, H. and Nakata, S. Increase of stratifin triggered by ultraviolet irradiation is possibly related to premature aging of human skin. *Exp Dermatol.* 23 Suppl 1:32-6 (2014).

62. Raaby, L., Otkjaer, K., Salvskov-Iversen, M.L., Johansen, C. and Iversen, L. A Characterization of the expression of 14-3-3 isoforms in psoriasis, basal cell carcinoma, atopic dermatitis and contact dermatitis. *Dermatol Reports.* 2(2):e14 (2010).

63. Epp, N., Furstenberger, G., Muller, K., de Juanes, S., Leitges, M., Hausser, I., et al. 12R-lipoxygenase deficiency disrupts epidermal barrier function. *J Cell Biol.* 177(1):173-82 (2007).

64. Lesniak, W. and Graczyk-Jarzynka, A. The S100 proteins in epidermis: Topology and function. *Biochim Biophys Acta.* 1850(12):2563-72 (2015).

65. Abtin, A., Eckhart, L., Mildner, M., Ghannadan, M., Harder, J., Schroder, J.M., et al. Degradation by stratum corneum proteases prevents endogenous RNase inhibitor from blocking antimicrobial activities of RNase 5 and RNase 7. *J Invest Dermatol.* 129(9):2193-201 (2009).

66. Hagens, G., Masouye, I., Augsburger, E., Hotz, R., Saurat, J.H. and Siegenthaler, G. Calcium-binding protein S100A7 and epidermal-type fatty acid-binding protein are associated in the cytosol of human keratinocytes. *Biochem J.* 339 (Pt 2):419-27 (1999).

67. Hagens, G., Roulin, K., Hotz, R., Saurat, J.H., Hellman, U. and Siegenthaler, G. Probable interaction between S100A7 and E-FABP in the cytosol of human keratinocytes from psoriatic scales. *Mol Cell Biochem.* 192(1-2):123-8 (1999).

68. Ogawa, E., Owada, Y., Ikawa, S., Adachi, Y., Egawa, T., Nemoto, K., et al. Epidermal FABP (FABP5) regulates keratinocyte differentiation by 13(S)-HODE-mediated activation of the NF-kappaB signaling pathway. *J Invest Dermatol.* 131(3):604-12 (2011).

69. Armstrong, E.H., Goswami, D., Griffin, P.R., Noy, N. and Ortlund, E.A. Structural basis for ligand regulation of the fatty acid-binding protein 5, peroxisome proliferator-activated receptor beta/delta (FABP5-PPARbeta/delta) signaling pathway. *J Biol Chem.* 289(21):14941-54 (2014).

70. Donovan, M., Ambach, A., Thomas-Collignon, A., Prado, C., Bernard, D., Jammayrac, O., et al. Calmodulin-like skin protein level increases in the differentiated epidermal layers in atopic dermatitis. *Exp Dermatol.* 22(12):836-7 (2013).

71. Mehul, B., Bernard, D., Brouard, M., Delattre, C. and Schmidt, R. Influence of calcium on the proteolytic degradation of the calmodulin-like skin protein (calmodulin-like protein 5) in psoriatic epidermis. *Exp Dermatol.* 15(6):469-77 (2006).

72. Sun, B.K., Boxer, L.D., Ransohoff, J.D., Siprashvili, Z., Qu, K., Lopez-Pajares, V., et al. CALML5 is a ZNF750- and TINCR-induced protein that binds stratifin to regulate epidermal differentiation. *Genes Dev.* 29(21):2225-30 (2015).

73. Bennett, R.D., Mauer, A.S., Pittelkow, M.R. and Strehler, E.E. Calmodulin-like protein upregulates myosin-10 in human keratinocytes and is regulated during epidermal wound healing in vivo. *J Invest Dermatol.* 129(3):765-9 (2009).

74. Grewal, T., Wason, S.J., Enrich, C. and Rentero, C. Annexins - insights from knockout mice. *Biol Chem.* 397(10):1031-53 (2016).

75. Watson, W.D., Srivastava, M., Leighton, X., Glasman, M., Faraday, M., Fossam, L.H., et al. Annexin 7 mobilizes calcium from endoplasmic reticulum stores in brain. *Biochim Biophys Acta.* 1742(1-3):151-60 (2004).

76. Kubler, M.D., Jordan, P.W., O'Neill, C.H. and Watt, F.M. Changes in the abundance and distribution of actin and associated proteins during terminal differentiation of human epidermal keratinocytes. *J Cell Sci.* 100 (Pt 1):153-65 (1991).

77. Eke Gungor, H., Sahiner, U.M., Karakukcu, C., Sahiner, N. and Altuner Torun, Y. The plasma gelsolin levels in atopic dermatitis: Effect of atopy and disease severity. *Allergol Immunopathol (Madr).* 44(3):221-5 (2016).

78. Lyukmanova, E.N., Shulepko, M.A., Kudryavtsev, D., Bychkov, M.L., Kulbatskii, D.S., Kasheverov, I.E., et al. Human Secreted Ly-6/uPAR Related Protein-1 (SLURP-1) Is a Selective Allosteric Antagonist of alpha7 Nicotinic Acetylcholine Receptor. *PLoS One.* 11(2):e0149733 (2016).

79. Arredondo, J., Chernyavsky, A.I., Jolkovsky, D.L., Webber, R.J. and Grando, S.A. SLURP-2: A novel cholinergic signaling peptide in human mucocutaneous epithelium. *J Cell Physiol.* 208(1):238-45 (2006).

80. Frezza, V., Terrinoni, A., Pitolli, C., Mauriello, A., Melino, G. and Candi, E. Transglutaminase 3 protects against photodamage. *J Invest Dermatol.* (2017).

81. Corradi, A., Franzi, A.T., Rubartelli, A. Synthesis and secretion of interleukin-1 alpha and interleukin-1 receptor antagonist during differentiation of cultured keratinocytes. *Exp Cell Res*. 217(2),355-62 (1995).

82. Hirao, T., Aoki, H., Yoshida, T., Sato, Y. and Kamoda, H. Elevation of interleukin 1 receptor antagonist in the stratum corneum of sun-exposed and ultraviolet B-irradiated human skin. *J Invest Dermatol.* 106(5):1102-7 (1996).

83. Ryu, J., Park, S.G., Park, B.C., Choe, M., Lee, K.S. and Cho, J.W. Proteomic analysis of psoriatic skin tissue for identification of differentially expressed proteins: up-regulation of GSTP1, SFN and PRDX2 in psoriatic skin. *Int J Mol Med.* 28(5):785-92 (2011).

84. Declercq, L., Sente, I., Hellemans, L., Corstjens, H. and Maes, D. Use of the synthetic superoxide dismutase/catalase mimetic EUK-134 to compensate for seasonal antioxidant deficiency by reducing pre-existing lipid peroxides at the human skin surface. *Int J Cosmet Sci.* 26(5):255-63 (2004).

85. Ishiwatari, S., Takahashi, M., Yasuda, C., Nakagawa, M., Saito, Y., Noguchi, N., et al. The protective role of DJ-1 in ultraviolet-induced damage of human skin:

DJ-1 levels in the stratum corneum as an indicator of antioxidative defense. *Arch Dermatol Res.* 307(10):925-35 (2015).

86. Kuehne, A., Emmert, H., Soehle, J., Winnefeld, M., Fischer, F., Wenck, H., et al. Acute Activation of Oxidative Pentose Phosphate Pathway as First-Line Response to Oxidative Stress in Human Skin Cells. *Mol Cell.* 59(3):359-71 (2015).

87. Schneider, E. and Ryan, T.J. Gamma-glutamyl hydrolase and drug resistance. *Clin Chim Acta.* 374(1-2):25-32 (2006).

86. Schallreuter-Wood, K.U., Pittelkow, M.R. and Swanson, N.N. Defective calcium transport in vitiliginous melanocytes. *Arch Dermatol Res.* 288(1):11-3 (1996).

89. Jayaprakash, P., Dong, H., Zou, M., Bhatia, A., O'Brien, K., Chen, M., et al. Hsp90alpha and Hsp90beta together operate a hypoxia and nutrient paucity stress-response mechanism during wound healing. *J Cell Sci.* 128(8):1475-80 (2015).

90. Murase, D., Hachiya, A., Fullenkamp, R., Beck, A., Moriwaki, S., Hase, T., et al. Variation in Hsp70-1A Expression Contributes to Skin Color Diversity. *J Invest Dermatol.* 136(8):1681-91 (2016).

91. Song, X., Narzt, M.S., Nagelreiter, I.M., Hohensinner, P., Terlecki-Zaniewicz, L., Tschachler, E., et al. Autophagy deficient keratinocytes display increased DNA damage, senescence and aberrant lipid composition after oxidative stress in vitro and in vivo. *Redox Biol.* 11:219-30 (2017).

92. Eggleton, P., Bremer, E., Dudek, E. and Michalak, M. Calreticulin, a therapeutic target? *Expert Opin Ther Targets.* 20(9):1137-47 (2016).

93. Mera, K., Kawahara, K., Tada, K., Kawai, K., Hashiguchi, T., Maruyama, I., et al. ER signaling is activated to protect human HaCaT keratinocytes from ER stress

induced by environmental doses of UVB. *Biochem Biophys Res Commun.* 397(2):350-4 (2010).

94. Kim, Y.I., Park, K., Kim, J.Y., Seo, H.S., Shin, K.O., Lee, Y.M., et al. An endoplasmic reticulum stress-initiated sphingolipid metabolite, ceramide-1-phosphate, regulates epithelial innate immunity by stimulating beta-defensin production. *Mol Cell Biol.* 34(24):4368-78 (2014).

95. Dahlmann, B. Mammalian proteasome subtypes: Their diversity in structure and function. *Arch Biochem Biophys.* 591:132-40 (2016).

96. Bulteau, A.L., Moreau, M., Nizard, C. and Friguet, B. Impairment of proteasome function upon UVA- and UVB-irradiation of human keratinocytes. *Free Radic Biol Med.* 32(11):1157-70 (2002).

97. Kuang, X.L., Liu, F., Chen, H., Li, Y., Liu, Y., Xiao, J., et al. Reductions of the components of the calreticulin/calnexin quality-control system by proteasome inhibitors and their relevance in a rodent model of Parkinson's disease. *J Neurosci Res.* 92(10):1319-29 (2014).

98. Sugiura, K. Unfolded protein response in keratinocytes: impact on normal and abnormal keratinization. *J Dermatol Sci.* 69(3):181-6 (2013).

99. Dahlqvist, J., Torma, H., Badhai, J. and Dahl, N. siRNA silencing of proteasome maturation protein (POMP) activates the unfolded protein response and constitutes a model for KLICK genodermatosis. *PLoS One.* 7(1):e29471 (2012).

100. Leo, M.S. and Sivamani, R.K. Phytochemical modulation of the Akt/mTOR pathway and its potential use in cutaneous disease. *Arch Dermatol Res.* 306(10):861-71 (2014).

101. Qin, L., Wang, Z., Tao, L. and Wang, Y. ER stress negatively regulates AKT/TSC/mTOR pathway to enhance autophagy. *Autophagy.* 6(2):239-47 (2010).

Gene name	Protein name	Fold change cheek / PA	p value	q value
1) Proteases				
NPEPPS	Puromycin-sensitive aminopeptidase	14.62	<0.001	<0.001
ASPRV1	Retroviral-like aspartic protease 1 (SASPase)	5.73	<0.001	<0.001
PRSS8	Prostasin	4.86	< 0.01	<0.001
KLK7	Kallikrein-7	4.27	<0.001	<0.001
CTSV	Cathepsin L2	3.96	<0.001	<0.001
DPP7	Dipeptidyl peptidase 2	3.49	< 0.05	< 0.01
CPA4	Carboxypeptidase A4	2.51	<0.001	<0.001
APEH	Acylamino-acid-releasing enzyme	2.46	<0.001	<0.001
NCSTN	Nicastrin	2.42	< 0.01	< 0.01
KLK10	Kallikrein-10	2.39	<0.001	<0.001
BLMH	Bleomycin hydrolase	1.73	<0.001	<0.001
PRCP	Lysosomal Pro-X carboxypeptidase	1.66	ns	ns
CASP14	Caspase-14	1.66	<0.001	<0.001
CTSD	Cathepsin D	1.43	<0.001	<0.001
CAPNS2	Calpain small subunit 2	1.40	ns	< 0.05
SCPEP1	Retinoid-inducible serine carboxypeptidase	1.26	ns	< 0.05
CAPN1	Calpain-1 catalytic subunit (calpain-1)	1.25	<0.001	<0.001
CTSB	Cathepsin B	1.24	< 0.05	< 0.01
CTSC	Dipeptidyl peptidase 1	1.18	ns	< 0.05
2) Protease inhibitors				
SERPINB7	Serpin B7	13.87	<0.001	<0.001
SERPINB5	Serpin B5	3.65	<0.001	<0.001
SERPINB13	Serpin B13	2.72	<0.001	<0.001
SERPINB3	Serpin B3	2.01	<0.001	<0.001
SERPINB8	Serpin B8	1.56	<0.001	<0.001
SERPINB4	Serpin B4	1.35	ns	< 0.05
SERPINB12	Serpin B12	1.28	ns	< 0.05
CSTA	Cystatin-A	0.85	< 0.05	< 0.01
A2ML1	Alpha-2-macroglobulin-like protein 1	0.67	< 0.01	<0.001
SERPINA12	Serpin A12	0.30	<0.001	<0.001
3) Corneodesmosome consistuents				
EPPK1	Epiplakin	3.78	**<0.001**	**<0.001**
JUP	Junction plakoglobin	2.63	<0.001	<0.001
PKP1	Plakophilin-1	2.59	<0.001	<0.001
POF1B	Protein POF1B	1.74	ns	< 0.05
DSP	Desmoplakin	1.61	<0.001	<0.001
DSG1	Desmoglein-1	1.49	< 0.01	< 0.01
CDSN	Corneodesmosin	1.20	<0.001	<0.001
DSC3	Desmocollin-3	1.19	< 0.01	< 0.01
4) Cornified envelope consitituents				
SPRR2B	Small proline-rich protein 2B	1.45	ns	ns
SPRR1B	Cornifin-B (small proline-rich protein IB)	1.26	ns	ns
CNFN	Cornifelin	0.74	< 0.05	< 0.01
SBSN	Suprabasin	0.43	<0.001	<0.001
SPRR2E	Small proline-rich protein 2E	0.22	<0.001	<0.001
SPRR1A	Cornifin-A (small proline-rich protein IA)	0.20	<0.001	<0.001
LOR	Loricrin	0.18	<0.001	<0.001
XP32	Skin-specific protein 32	0.16	<0.001	<0.001

5) Cornified envelope processing enzymes				
TGM3	Protein-glutamine gamma-glutamyltransferase E (transglutaminase-3)	1.64	<0.001	<0.001
TGM1	Protein-glutamine gamma-glutamyltransferase K (transglutaminase-1)	1.47	< 0.01	<0.001
ALOXE3	Hydroperoxide isomerase ALOXE3, epidermis-type lipoxygenase 3 (eLOX)	1.02	ns	ns
ALOX12B	Arachidonate 12-lipoxygenase, 12R-type (12R-LOX)	0.31	<0.001	<0.001
6) Filaggrin and its processing enzymes				
FLG	Filaggrin	3.08	<0.001	<0.001
ARG1	Arginase-1	3.05	<0.001	<0.001
HAL	Histidine ammonia-lyase	2.85	< 0.01	<0.001
FLG2	Filaggrin-2	1.12	ns	< 0.05
GGCT	Gamma-glutamylcyclotransferase	1.10	ns	< 0.05
7) Keratins				
KRT39	Keratin, type I cytoskeletal 39	4.68	<0.001	<0.001
KRT5	Keratin, type II cytoskeletal 5	1.67	<0.001	<0.001
KRT82	Keratin, type II cuticular Hb2	1.62	< 0.05	< 0.01
KRT6C	Keratin, type II cytoskeletal 6C	1.57	ns	< 0.05
KRT2	Keratin, type II cytoskeletal 2 epidermal	1.50	<0.001	<0.001
KRT16	Keratin, type I cytoskeletal 16	1.46	<0.001	<0.001
KRT23	Keratin, type I cytoskeletal 23	1.45	< 0.05	< 0.01
KRT14	Keratin, type I cytoskeletal 14	1.40	<0.001	<0.001
KRT1	Keratin, type II cytoskeletal 1	0.86	<0.001	<0.001
KRT10	Keratin, type I cytoskeletal 10	0.79	<0.001	<0.001
KRT86	Keratin, type II cuticular Hb6	0.77	ns	< 0.05
KRT32	Keratin, type I cuticular Ha2	0.69	< 0.01	<0.001
KRTAP3-1	Keratin-associated protein 3-1	0.68	ns	< 0.05
KRTAP3-2	Keratin-associated protein 3-2	0.65	ns	< 0.05
KRT80	Keratin, type II cytoskeletal 80	0.61	<0.001	<0.001
KRT38	Keratin, type I cuticular Ha8	0.59	< 0.05	< 0.01
KRT35	Keratin, type I cuticular Ha5	0.48	<0.001	<0.001
KRT17	Keratin, type I cytoskeletal 17	0.46	<0.001	<0.001
KRTAP2-1	Keratin-associated protein 2-1	0.42	< 0.01	<0.001
KRTAP24-1	Keratin-associated protein 24-1	0.41	ns	< 0.05
KRT34	Keratin, type I cuticular Ha4	0.36	<0.001	<0.001
KRT33B	Keratin, type I cuticular Ha3-II	0.36	<0.001	<0.001
KRT31	Keratin, type I cuticular Ha1	0.25	<0.001	<0.001
KRT6B	Keratin, type II cytoskeletal 6B	0.25	<0.001	<0.001
KRT78	Keratin, type II cytoskeletal 78	0.12	<0.001	<0.001
8) Calcium binding proteins / AMPs				
CALML3	Calmodulin-like protein 3	2.78	<0.001	<0.001
S100A16	Protein S100-A16	1.85	<0.001	<0.001
CALML5	Calmodulin-like protein 5	1.52	< 0.05	< 0.01
S100A14	Protein S100-A14	0.86	ns	< 0.05
S100A8	Protein S100-A8	0.60	<0.001	<0.001
S100A9	Protein S100-A9	0.49	<0.001	<0.001
S100A7	Protein S100-A7 (Psoriasin)	0.48	<0.001	<0.001
S100A3	Protein S100-A3	0.08	<0.001	<0.001
9) Glycoprotein folding protein				
CALR	Calreticulin	0.45	ns	< 0.05
10) Annexins				

ANXA7	Annexin A7	9.18	<0.001	<0.001
ANXA11	Annexin A11	5.09	< 0.05	< 0.01
ANXA1	Annexin A1	1.79	< 0.05	< 0.01
11) Inflammation-associated proteins				
IL1RN	Interleukin-1 receptor antagonist protein	107.67	<0.001	<0.001
IGHA1	Ig alpha-1 chain C region	17.11	<0.001	<0.001
SLURP1	Secreted Ly-6/uPAR-related protein 1	5.64	<0.001	<0.001
LYPD3	Ly6/PLAUR domain-containing protein 3	4.01	<0.001	<0.001
IGLC3	Ig lambda-3 chain C regions	1.92	< 0.05	< 0.01
IGKC	Ig kappa chain C region	1.59	<0.001	<0.001
IL36G	Interleukin-36 gamma	1.49	<0.001	<0.001
HLA-DRA	HLA class II histocompatibility antigen, DR alpha chain	1.20	ns	ns
IGHG4	Ig gamma-4 chain C region	0.70	ns	< 0.05
IGHG1	Ig gamma-1 chain C region	0.19	<0.001	<0.001
12) Microtubules / tubulins				
TUBA4A	Tubulin alpha-4A chain	6.66	<0.001	<0.001
TUBA1B	Tubulin alpha-1B chain	1.37	< 0.05	< 0.01
13) Membrane trafficking / myosin				
MYH9	Myosin-9	2.83	<0.001	<0.001
MYH14	Myosin-14	2.12	< 0.05	< 0.01
TPM3	Tropomyosin alpha-3 chain	1.19	ns	< 0.05
TPM4	Tropomyosin alpha-4 chain	0.52	ns	< 0.05
CLTC	Clathrin heavy chain 1	0.29	< 0.05	< 0.01
14) Actin binding proteins / cytoskeleton				
GSN	Gelsolin	60.67	<0.001	<0.001
AHNAK	Neuroblast differentiation-associated protein AHNAK	44.29	<0.001	<0.001
PLEC	Plectin	31.90	<0.001	<0.001
CFL1	Cofilin-1	9.07	<0.001	<0.001
ACTN4	Alpha-actinin-4	5.05	<0.001	<0.001
FLNB	Filamin-B	3.66	<0.001	<0.001
LCP1	Plastin-2	1.32	< 0.05	< 0.01
ACTG1	Actin, cytoplasmic 2	0.36	<0.001	<0.001
15) Antimicrobial proteins				
RNH1	Ribonuclease inhibitor	13.39	<0.001	<0.001
DCD	Dermcidin	0.76	ns	< 0.05
LYG2	Lysozyme g-like protein 2	0.61	ns	< 0.05
16) Proteasome				
PSMB1	Proteasome subunit beta type-1	6.14	<0.001	<0.001
PSMB3	Proteasome subunit beta type-3	5.78	<0.001	<0.001
PSMA2	Proteasome subunit alpha type-2	5.14	<0.001	<0.001
PSMA5	Proteasome subunit alpha type-5	2.47	<0.001	<0.001
PSMA1	Proteasome subunit alpha type-1	2.12	<0.001	<0.001
PSMB2	Proteasome subunit beta type-2	1.60	<0.001	<0.001
PSMA3	Proteasome subunit alpha type-3	1.42	<0.001	<0.001
PSMA6	Proteasome subunit alpha type-6	1.23	< 0.05	< 0.01
PSMA7	Proteasome subunit alpha type-7	1.22	ns	< 0.05
RPS27A	Ubiquitin-40S ribosomal protein S27a	1.22	ns	< 0.05
PSMB6	Proteasome subunit beta type-6	0.44	<0.001	<0.001
17) Protein folding control				
HSPA5	78 kDa glucose-regulated protein	3.66	<0.001	<0.001
18) Antioxidant related proteins				
GSTP1	Glutathione S-transferase P	7.78	<0.001	<0.001

SELENBP1	Selenium-binding protein 1	3.85	<0.001	<0.001
GLRX	Glutaredoxin-1	3.20	<0.001	<0.001
PRDX2	Peroxiredoxin-2	3.14	<0.001	<0.001
ME1	NADP-dependent malic enzyme	2.37	<0.001	<0.001
GGH	Gamma-glutamyl hydrolase	2.21	<0.001	<0.001
ALDH7A1	Alpha-aminoadipic semialdehyde dehydrogenase	1.94	< 0.05	< 0.01
PRDX6	Peroxiredoxin-6	1.59	ns	ns
SOD1	Superoxide dismutase [Cu-Zn]	1.38	ns	< 0.05
PARK7	Protein DJ-1	1.35	< 0.05	< 0.01
TXN	Thioredoxin	1.17	< 0.05	< 0.01
PRDX1	Peroxiredoxin-1	1.15	ns	< 0.05
CAT	Catalase	0.85	ns	< 0.05
19) Heat shock proteins				
HSPA1A	Heat shock 70 kDa protein 1A/1B	1.98	< 0.01	<0.001
HSPB1	Heat shock protein beta-1	1.23	< 0.05	< 0.01
HSP90AA1	Heat shock protein HSP 90-alpha	0.06	<0.001	<0.001
20) Signal transduction proteins				
SFN	14-3-3 protein sigma	19.83	<0.001	<0.001
NPM1	Nucleophosmin	4.34	<0.001	<0.001
C1QBP	Complement component 1 Q subcomponent-binding protein, mitochondrial	4.29	<0.001	<0.001
YWHAZ	14-3-3 protein zeta/delta	2.36	< 0.01	<0.001
CREG1	Protein CREG1	2.13	<0.001	<0.001
BANF1	Barrier-to-autointegration factor	1.80	< 0.05	< 0.01
LMNA	Prelamin-A/C	1.36	ns	< 0.05
CAMK2D	Calcium/calmodulin-dependent protein kinase type II subunit delta	1.32	ns	ns
ECM1	Extracellular matrix protein 1	1.17	< 0.01	<0.001
GSDMA	Gasdermin-A	1.17	ns	< 0.05
KPRP	Keratinocyte proline-rich protein	0.90	ns	< 0.05
21) SC lipid metabolism / transport proteins / enzymes				
PLBD1	Phospholipase B-like 1	4.29	<0.001	<0.001
GBA	Glucosylceramidase	3.75	<0.001	<0.001
LCN1	Lipocalin-1	2.00	<0.001	<0.001
ASAH1	Acid ceramidase	1.50	< 0.01	<0.001
PSAPL1	Proactivator polypeptide-like 1	1.18	ns	< 0.05
APOD	Apolipoprotein D	0.59	<0.001	<0.001
FABP5	Fatty acid-binding protein, epidermal	0.57	<0.001	<0.001
PSAP	Prosaposin	0.32	<0.001	<0.001
HSD17B4	Peroxisomal multifunctional enzyme type 2	0.26	<0.001	<0.001
22) Nucleotide metabolism				
TYMP	Thymidine phosphorylase	2.31	<0.001	<0.001
IMPA2	Inositol monophosphatase 2	0.62	<0.001	<0.001
CDA	Cytidine deaminase	0.21	<0.001	<0.001
23) Secreted proteins				
LGALS7	Galectin-7	1.13	ns	< 0.05
LGALS3	Galectin-3	0.68	ns	< 0.05
24) Lysosomal proteins				
ACPP	Prostatic acid phosphatase	14.15	<0.001	<0.001
C11orf54	Ester hydrolase C11orf54	9.40	<0.001	<0.001
LAMP1	Lysosome-associated membrane glycoprotein 1	6.96	<0.001	<0.001
NEU2	Sialidase-2	2.74	<0.001	<0.001

GAA	Lysosomal alpha-glucosidase	2.65	< 0.01	<0.001
LAMP2	Lysosome-associated membrane glycoprotein 2	1.62	<0.001	<0.001
CTSA	Lysosomal protective protein	1.30	< 0.05	< 0.01
GM2A	Ganglioside GM2 activator	0.50	<0.001	<0.001
25) Glycolysis / Krebs cycle enzymes				
MDH2	Malate dehydrogenase, mitochondrial	16.28	<0.001	<0.001
ENO1	Alpha-enolase	6.31	<0.001	<0.001
TPI1	Triosephosphate isomerase	6.01	<0.001	<0.001
GAPDH	Glyceraldehyde-3-phosphate dehydrogenase	2.73	<0.001	<0.001
PKM	Pyruvate kinase PKM	1.21	ns	< 0.05
ATP5B	ATP synthase subunit beta, mitochondrial	1.68	ns	< 0.05
26) Serum diffusion markers				
VCP	Transitional endoplasmic reticulum ATPase	31.86	<0.001	<0.001
ALB	Serum albumin	0.60	<0.001	<0.001
27) Protein synthesis				
SSR1	Translocon-associated protein subunit alpha	4.43	<0.001	<0.001
EIF6	Eukaryotic translation initiation factor 6	3.00	<0.001	<0.001
EEF2	Elongation factor 2	0.44	< 0.01	<0.001
28) Aminoacid /purine / pyrimidine metabolism				
GLUL	Glutamine synthetase	3.78	<0.001	<0.001
NUDT5	ADP-sugar pyrophosphatase	3.19	<0.001	<0.001
GPT	Alanine aminotransferase 1	1.95	ns	< 0.05
29) Retinoid metabolism				
SDR9C7	Short-chain dehydrogenase/reductase family 9C member 7	1.52	< 0.01	< 0.01