

# Mass spectrometry-based proteomics of facial stratum corneum of different ethnic groups to reveal novel biochemical pathways involved in photodamaged dry skin

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## Introduction

Although our understanding of the ethnic differences in stratum corneum (SC) structure, composition and function has increased tremendously over the last few decades, our knowledge regarding effects of photoaging on SC composition is minimal, although differences in natural moisturizing factor (NMF) levels and zonal skin hydration are clearly apparent.

We have previously shown variations in the biochemistry and physiology of facial SC among Albino Africans, Black Africans and Caucasians (Table I). Despite having higher NMF levels, implying healthier skin, Albino Africans have elevated basal TEWL and drier skin. This seems to be related to increased presence of immature corneocytes. Thus, our aim was to utilize proteomics to understand the effects of photodamage on facial SC (Figure 1) and to elucidate variations in the filaggrinolytic pathway (Figure 2) among differently pigmented ethnic groups.



Figure 3. Representative images of Albino African, Black African and Caucasian subjects.

Parameter	Albino Africans	Black Africans	Caucasians	p values of comparison		
				A vs B	A vs C	B vs C
Expert grading, facial dryness	1.9 ± 0.2	0.9 ± 0.1	1.1 ± 0.1	<0.001	<0.005	n.s.
Expert grading, facial roughness	2.1 ± 0.2	1.1 ± 0.1	1.3 ± 0.1	<0.001	<0.005	n.s.
Capacitance [AU], cheek	34.0 ± 3.1	52.9 ± 2.2	40.2 ± 2.8	<0.001	n.s.	<0.01
Capacitance [AU], PA	25.0 ± 2.4	41.1 ± 2.1	32.5 ± 2.5	<0.001	n.s.	<0.005
Basal TEWL [g m <sup>-2</sup> h <sup>-1</sup> ], cheek	19.5 ± 1.5	10.3 ± 0.8	11.8 ± 0.8	<0.001	<0.001	n.s.
Basal TEWL [g m <sup>-2</sup> h <sup>-1</sup> ], PA	17.8 ± 1.3	10.8 ± 2.4	9.8 ± 0.6	<0.001	<0.001	n.s.
Skin surface pH, cheek	5.93 ± 0.09	5.68 ± 0.10	5.67 ± 0.09	n.s.	n.s.	n.s.
PCA [μmol mg <sup>-1</sup> SC protein], cheek	0.50 ± 0.07	0.17 ± 0.03	0.094 ± 0.01	<0.001	<0.001	<0.01
PCA [μmol mg <sup>-1</sup> SC protein], PA	0.44 ± 0.05	0.15 ± 0.02	0.070 ± 0.01	<0.001	<0.001	<0.001
CE maturity, cheek	0.59 ± 0.02	0.83 ± 0.02	0.81 ± 0.02	<0.001	<0.001	n.s.
CE maturity, PA	0.65 ± 0.02	0.93 ± 0.04	0.93 ± 0.04	<0.001	<0.001	n.s.

Table I. Comparison of expert grading, biophysical data, PCA (NMF) levels and cornified envelope maturity (ratio of differential Nile red and involucrin immunostaining). Post-auricular, PA; data are mean ± SEM; n.s., not significant.

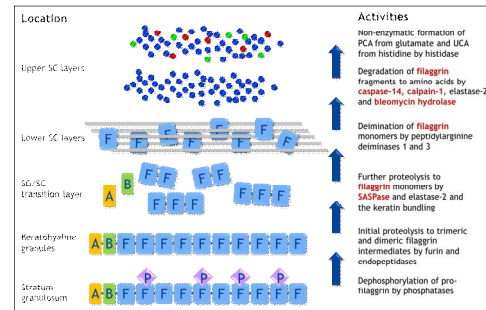


Figure 2. Processing of pro-filaggrin in terminal epidermal differentiation. Proteins found in our approach are highlighted in red font.

## Methods

Eighteen female subjects of different skin ethnicities, living in Pretoria, South Africa were enrolled. The panel consisted of Albino Africans (40.8±6.2 years), Black Africans (43.2±4.1 years) and Caucasians (39.0±5.3 years) (Figure 3). Their photodamaged cheek and photoprotected post-auricular (PA) SC were investigated (Figure 4).

On both test sites, nine subsequent tape strippings were taken and extracted by sonication in PBS buffer containing SDS and anti-proteases. Urea and TRIS-HCl buffer soluble proteins were trypsinized and separated using a nanoACQUITY UPLC Symmetry C18 Trap Column, 180 μm x 20 mm in trap and elute mode with ACQUITY UPLC Peptide BEH C18 nanoACQUITY Column 75 μm x 250 mm by a Eksigent Ultra Plus nano-LC 2D HPLC coupled to a TripleTOF<sup>®</sup> 5600 mass spectrometer interfaced to a nano spray II source. DDA spectra processing and database searching was performed with ProteinPilot (v4.5 beta, ABSciex, Framingham) using the Paragon algorithm.

## Affiliations

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Total SC proteins identified  
Cheek (photoexposed): 473  
PA (photoprotected): 253  
Proteins related to SC maturation  
Cheek (photoexposed): 57  
PA (photoprotected): 43



Figure 1. Number of facial SC proteins identified by proteomics

Figure 4. Location of tape strippings on photoexposed cheek and photoprotected post-auricular sites.

## Results

### Cheek, interethnic comparison

Filaggrin, filaggrin-2, bleomycin hydrolase and calpain-1 levels were highest for the Caucasian and lowest for the Albino African subjects (Table IIa and Figure 5). The level of the upstream, skin-specific retroviral-like aspartic protease (SASPase) was slightly higher in Albino African compared to Black African subjects. No significant differences for any of the proteins were found between Black African and Caucasian subjects.

### Post-auricular site, interethnic comparison

As on the cheek, filaggrin-2 levels were highest in the Caucasian and lowest in the Albino African subjects (Table IIb and Figure 5). Conversely, filaggrin levels were highest in the Black African subjects. Bleomycin hydrolase and caspase-14 levels were similar across the three ethnic groups but Albino Africans had significantly lower levels of calpain-1. Albino Africans had a slightly higher levels of SASPase compared with the other two ethnic groups. Like on the cheek, no significant differences for any of the proteins were found between Black African and Caucasian subjects.

### Cheek vs post-auricular site, intraethnic comparison

Most of the proteins were significantly upregulated on the photoexposed site, although filaggrin-2, calpain-1 and caspase-14 were only marginally elevated in all three groups. SASPase levels were significantly increased by factor >3 in all three ethnicities and filaggrin in Albino Africans and Caucasians (Table IIc and Figure 5).

## Conclusion

Presumably because of UV irradiation, the levels of filaggrin proteins and associated processing enzymes are increased on photoexposed facial skin in all three ethnic groups. Nevertheless, the resulting elevated NMF levels are insufficient to correct the underlying biochemical abnormality in corneocyte maturation, particularly in the Albino African subjects. The lower levels of filaggrin-2 may be associated with these conditions.

Our results demonstrate that the increased levels of filaggrin proteins, their processing enzymes and the resulting NMF are insufficient to correct the cellular and biochemical abnormalities in photodamaged skin.

A better understanding of the impact of filaggrin-2 on epidermal differentiation *in vivo* is needed.

## Acknowledgements

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### a) Comparison A vs B vs C, Cheek

Protein names	Gene names	Cheek					
		A vs B			A vs C		
		p-value	q-value	Fold change	p-value	q-value	Fold change
Filaggrin	FLG	0.824	0.619	0.87	0.520	0.540	0.68
Filaggrin-2	FLG2	0.000	0.002	0.52	0.000	0.000	0.37
Bleomycin hydrolase	BLMH	0.459	0.515	0.78	0.341	0.449	0.73
Calpain-1	CAPN1	0.026	0.101	0.67	0.001	0.009	0.56
Caspase-14	CASP14	0.953	0.646	0.99	0.511	0.537	0.85
SASPase	ASPRV1	0.381	0.470	1.49	0.677	0.580	0.83

### b) Comparison A vs B vs C, PA

Protein names	Gene names	Postauricular					
		A vs B			A vs C		
		p-value	q-value	Fold change	p-value	q-value	Fold change
Filaggrin	FLG	0.033	0.067	0.31	0.420	0.351	0.64
Filaggrin-2	FLG2	0.000	0.000	0.32	0.000	0.000	0.22
Bleomycin hydrolase	BLMH	0.643	0.443	0.91	0.129	0.174	0.74
Calpain-1	CAPN1	0.000	0.000	0.63	0.000	0.000	0.54
Caspase-14	CASP14	0.413	0.348	1.13	0.972	0.545	1.01
SASPase	ASPRV1	0.138	0.184	1.74	0.294	0.289	1.48

### c) Comparison Cheek vs PA

Protein names	Gene names	Albino Africans			Black Africans			Caucasians		
		Cheek vs PA			Cheek vs PA			Cheek vs PA		
		p-value	q-value	Fold change	p-value	q-value	Fold change	p-value	q-value	Fold change
Filaggrin	FLG	0.000	0.000	3.27	0.665	0.106	1.15	0.000	0.000	3.08
Filaggrin-2	FLG2	0.000	0.000	1.89	0.120	0.023	1.17	0.267	0.047	1.12
Bleomycin hydrolase	BLMH	0.000	0.000	1.70	0.000	0.000	1.98	0.000	0.000	1.73
Calpain-1	CAPN1	0.000	0.000	1.28	0.006	0.001	1.19	0.000	0.000	1.25
Caspase-14	CASP14	0.000	0.000	1.40	0.000	0.000	1.61	0.000	0.000	1.66
SASPase	ASPRV1	0.000	0.000	3.20	0.000	0.000	3.73	0.000	0.000	5.73

Table II. Comparison of filaggrin, filaggrin-2 and processing enzyme ratios in sun-exposed cheek and sun-protected post-auricular (PA) SC. Blue areas, p/q<0.05; red areas, fold change <0.5; green areas, fold change >2.0. A, Albino Africans; B, Black Africans; C, Caucasians.

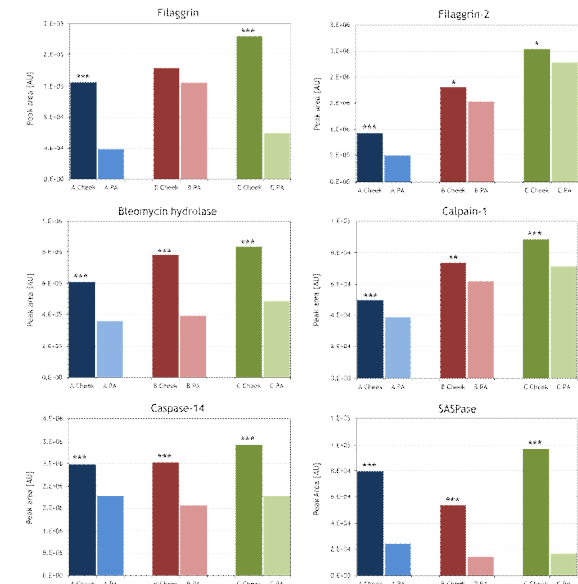


Figure 5. Comparison of filaggrin, filaggrin-2 and processing enzyme levels in sun-exposed cheek and sun-protected post-auricular (PA) SC. A, Albino Africans; B, Black Africans; C, Caucasians. \* q<0.05, \*\* q<0.01, \*\*\* q<0.001.