An investigation of protease activity at non-lesional sites in Atopic Dermatitis

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INTRODUCTION

- Elevated protease activity within the stratum corneum (SC) accelerates barrier breakdown in chronic skin diseases such as atopic dermatitis (AD).[1]
- We have developed a simple, non-invasive assay to quantify a broad spectrum of proteases at the skin surface.
- Using this assay, we recently identified a subset of neonates at-risk of developing AD with elevated protease activity, highlighting the potential for protease-associated barrier breakdown in normal appearing skin.[2]
- To extend these finding we have investigated protease activity at non-lesional sites in AD.

AIMS

1. To assess surface protease activity in conjunction with the biophysical and molecular properties of the skin barrier in AD patients at non-lesional sites.
2. To place our AD findings into context by comparing the results to a healthy adult cohort.

METHODS

Subjects

- **AD cohort:** Patients with quiescent AD (*n*=20) and active disease (*n*=68) underwent skin assessments at a single visit. A diagnosis of AD was made in accordance with the UK working party diagnostic criteria.[3]
- **Healthy adult cohort:** For comparison a cohort of adults with no history of skin disease or atopy was recruited and underwent identical assessments (*n*=20).

Biophysical measurements

- Permeability barrier function was determined by measuring transepidermal water loss (TEWL) using an AquaFlux evaporimeter (Biox, UK) in climate controlled conditions.[4]
- Skin surface pH and SC hydration was determined using a Skin-pH-meter and Corneometer (C&K, Germany).[5,6]
- Tape stripping was combined with IR densitometry (Heiland Electronic, Wetzlar, Germany) to measure mass of SC removed.[7]

Protease activity

- A broad-spectrum casein substrate was used to assay surface protease activity on forearm-collected D-Squame discs in line with previously published methodology.[1]

ATR-FTIR spectroscopy

- Lipid structure (full width half maximum [FWHM50] 1480-1460cm^{-1})[8] was analysed *in-vivo* using a silver halide probe (Art Photonics, Berlin, Germany) attached to a Nicolet iS50 FTIR spectrometer (Thermo Fisher Scientific, Waltham, USA).[9]

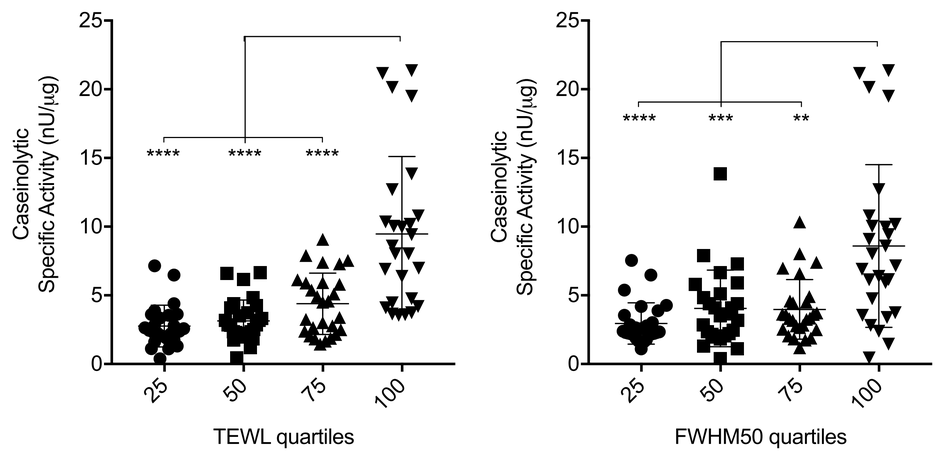
Genotyping

- Both cohorts were screened for the following AD risk loci:[10-12] ***FLG*:** R501X; 2282del4; R2447X; S3247X; 3702delG (Mentype® PCR kit, Biotype Diagnostic GmbH, Dresden, Germany) ***SPINK5*:** E420K (TaqMan™, Thermo Fisher Scientific, Waltham, USA) and **KLK7:** 3'UTR AACC insertion (SCCE).[13]

RESULTS

Elevated surface protease activity is associated with barrier dysfunction in AD

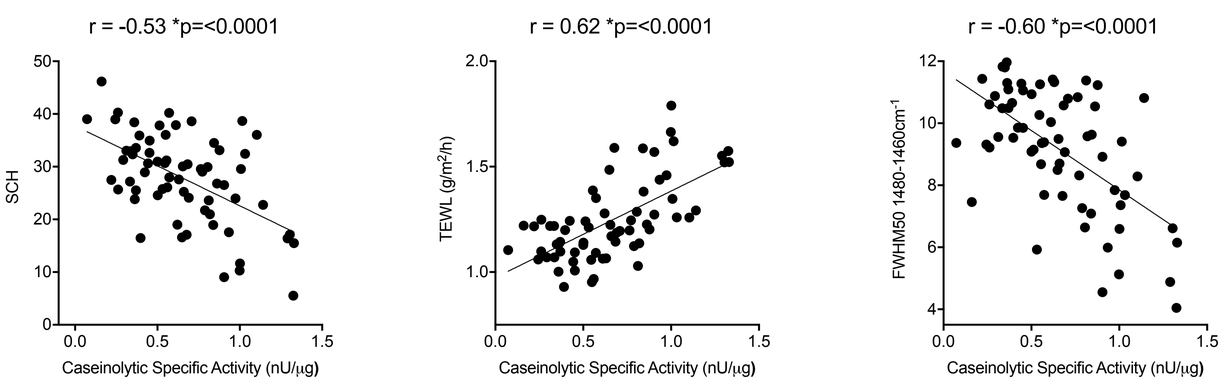
	Quiescent AD	*P* value	Healthy skin	*P* value	Active AD
Age (years)	29.1 (±11.5)		25.0 (±6.7)		27.0 (±12.0)
Sex (% male)	25		25		35
SCORAD (objective)	-		-		22.64 (±15.6)
Protease activity (nU/μg^{-1})	4.13 (±2.0)	ns	2.88 (±1.8)	*0.015	5.69 (±4.7)
TEWL (g/m^2/h)	14.6 (±2.9)	*0.0007	10.7 (±2.6)	*0.0001	19.3 (±10.3)
SC Hydration (RCU)	32.3 (±8.1)	ns	32.0 (±7.1)	ns	28.0 (±8.4)
Skin-surface pH	4.7 (±0.2)	ns	4.8 (±0.4)	ns	4.9 (±0.3)
Lipid structure (FWHM50)	9.6 (±2.0)	ns	11.0 (±1.2)	*0.0007	9.1 (±1.9)
***FLG* (% carriers)**	10		5		37
***SPINK5* (% carriers)**	50		65		80
***SCCE* (% carriers)**	65		55		46
Atopy (%)	45		0		63
Early onset AD (%)	45		-		50



Panel 1: Cohort demographics, (left) and the relationship between surface protease activity and TEWL / lipid structure determined by ATR-FTIR (right). Protease activity was normalised relative to protein mass quantified by IR densitometry. Significance was determined using a 1-way analysis of variance with Bonferroni's post-hoc analysis (****p=<0.0001). Mean ± standard deviation presented.

Surface protease activity correlates with SC hydration, TEWL and lipid structure

Panel 2: The relationship between surface protease activity and (a) stratum corneum (SC) hydration, (b) TEWL and (c) lipid structure (FWHM50) in patients with active disease at non-lesional sites. Pearson correlation coefficient calculated (r) and presented



No association of surface protease activity with *FLG, SPINK5, KLK7*, atopy and early onset AD

Protease activity (nU/μg^{-1})	Present	Not present	*P* value
Early onset AD	6.2 (±5.4)	5.2 (±4.0)	ns
Hayfever	5.7 (±5.6)	5.6 (±4.2)	ns
Asthma	6.1 (±5.0)	5.5 (±4.7)	ns
Food allergy	5.0 (±4.5)	6.0 (±5.0)	ns
***FLG* mutation**	6.5 (±5.9)	5.2 (±4.0)	ns
***SPINK5* mutation**	5.3 (±4.1)	7.3 (±7.0)	ns
***SCCE* mutation**	5.9 (±5.4)	5.5 (±4.3)	ns

Protease activity (nU/μg^{-1})	Early onset AD	Hayfever	Asthma	Food allergy	*FLG* mutation	*SPINK5* mutation	*SCCE* mutation
Early onset AD	-	6.6 (±6.5)	6.8 (±6.0)	5.9 (±5.3)	7.9 (±7.0)	6.0 (±5.1)	7.5 (±6.7)
Hayfever	6.6 (±6.5)	-	6.1 (±5.9)	4.8 (±4.8)	7.3 (±6.8)	5.6 (±5.2)	7.7 (±7.4)
Asthma	6.8 (±6.0)	6.1 (±5.9)	-	5.8 (±5.0)	6.9 (±6.7)	5.7 (±4.2)	8.5 (±6.1)
Food allergy	5.9 (±5.3)	4.8 (±4.8)	5.8 (±5.0)	-	4.9 (±5.5)	4.8 (±4.4)	5.4 (±4.9)
***FLG* mutation**	7.9 (±7.0)	7.3 (±6.8)	6.9 (±6.7)	4.9 (±5.5)	-	6.4 (±5.6)	8.2 (±8.4)
***SPINK5* mutation**	6.0 (±5.1)	5.6 (±5.2)	5.7 (±4.2)	4.8 (±4.4)	6.4 (±5.6)	-	5.5 (±5.0)
***SCCE* mutation**	7.5 (±6.7)	7.7 (±7.4)	8.5 (±6.1)	5.4 (±4.9)	8.2 (±8.4)	5.5 (±5.0)	-

Panel 3: Effect of AD risk alleles, atopy and early disease onset on surface protease activity in patients with active disease. Significance was determined using an unpaired students t-test. Mean ± standard deviation presented.

CONCLUSIONS

- Subjects with quiescent AD possess a significant permeability barrier function defect compared to healthy controls with no history of atopy.
- In patients with active AD, protease activity is significantly elevated at both lesional[1] and non-lesional sites.
- This elevation in protease activity was associated with a less orthorhombic SC lipid structure and weakened permeability barrier function providing new insight to barrier breakdown in non-lesional skin.
- *FLG*[10], *SPINK5*[11] and *KLK7*[12] AD risk alleles did not confer elevated protease activity at the skin surface in patients with active disease.

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