Abnormal corneodesmosome distribution in atopic dermatitis and soap-induced xerosis

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INTRODUCTION

- Serine proteases drive stratum corneum (SC) desquamation through the proteolysis of corneodesmosomes[1,2].
- Two key corneodesmosome adhesion proteins, desmoglein 1 and desmocollin 1, are degraded by trypsin-like and chymotrypsin-like serine proteases to facilitate corneocyte sloughing[3].
- In the lower SC, the *stratum compactum*, corneodesmosomes cover the corneocyte surface uniformly. As corneocytes mature and progress upwards through the SC, the corneodesmosomes become degraded. In the uppermost layers of the SC, the *stratum disjunctum*, only the peripheral corneodesmosomes remain[4].
- SC pH is an important regulator of skin barrier homeostasis, and when elevated increases the activity of serine proteases, which in turn accelerates corneodesmosome proteolysis, resulting in barrier breakdown[5,6].
- We aimed to investigate the consequences of elevated SC protease activity in atopic dermatitis (AD) and soap-induced xerosis at the SC surface.

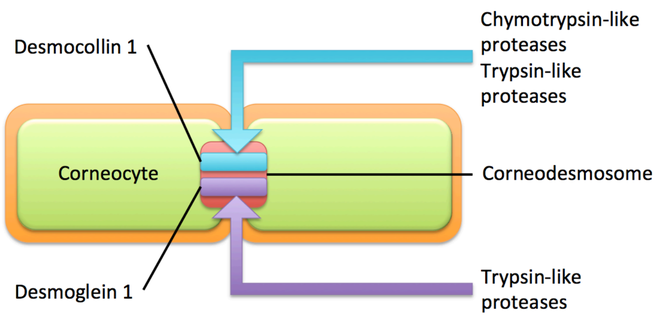

Figure 1: Cell-cell adhesion of corneocytes

Aim: To investigate the functional consequences of elevated proteases in the skin.

METHODS

2 cohorts were recruited. Cohort 1 contained a non-atopic control group (n=12) and an active AD group (n=12). Cohort 2 was non-atopic and underwent an exaggerated washing regimen to induce xerosis on one forearm using an alkyl carboxylate soap, leaving the other forearm untreated (n=5).

- Desmoglein 1 and desmocollin 1 distribution was determined for samples of SC removed by tape stripping. Samples were stained with anti-DSG1 (Progen Biotechnik) or anti-DSC1 (Santa Cruz Biotechnology) and Alexa Fluor 488 (Life Technologies). Samples were observed using an inverted Zeiss LSM 510 NLO confocal microscope, and scored using Image J (see **Figure 2**).
- SC caseinolytic, trypsin-like and chymotrypsin-like protease activity was determined for samples of SC removed by tape stripping[7].
- Skin-surface-pH was measured using a Skin-pH-meter PH905 (C&K, Germany).
- Skin barrier function was measured using an AquaFlux TEWL machine (Biox, UK).
- Corneocyte size was determined by measuring the surface area of fluorescently stained corneocytes using Image J.
- SC thickness and cohesion was determined by removing the SC by tape stripping and measuring protein content[8].
- Statistical significance was determined using GraphPad Prism 6 (ns $p>0.05$, * $p\leq0.05$, ** $p\leq0.01$, *** $p\leq0.001$, **** $p\leq0.0001$). Error bars on figures indicate SEM.

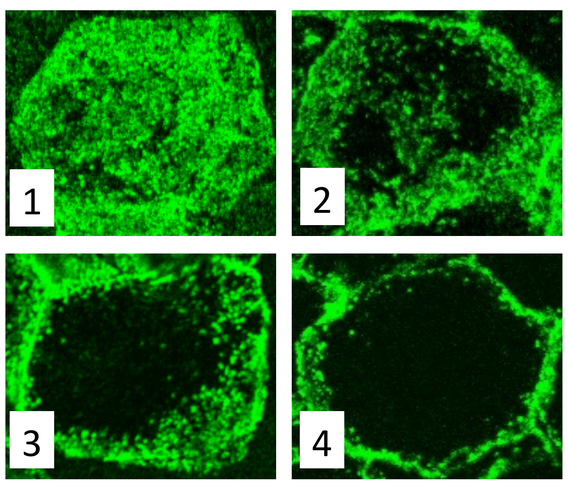

Figure 2: Desmoglein 1 and Desmocollin 1 distribution across each corneocyte was scored on a scale of 1 (uniform) to 4 (peripheral).

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RESULTS

Corneodesmosome distribution was more uniform in AD and more peripheral in soap-induced xerosis at the SC surface.

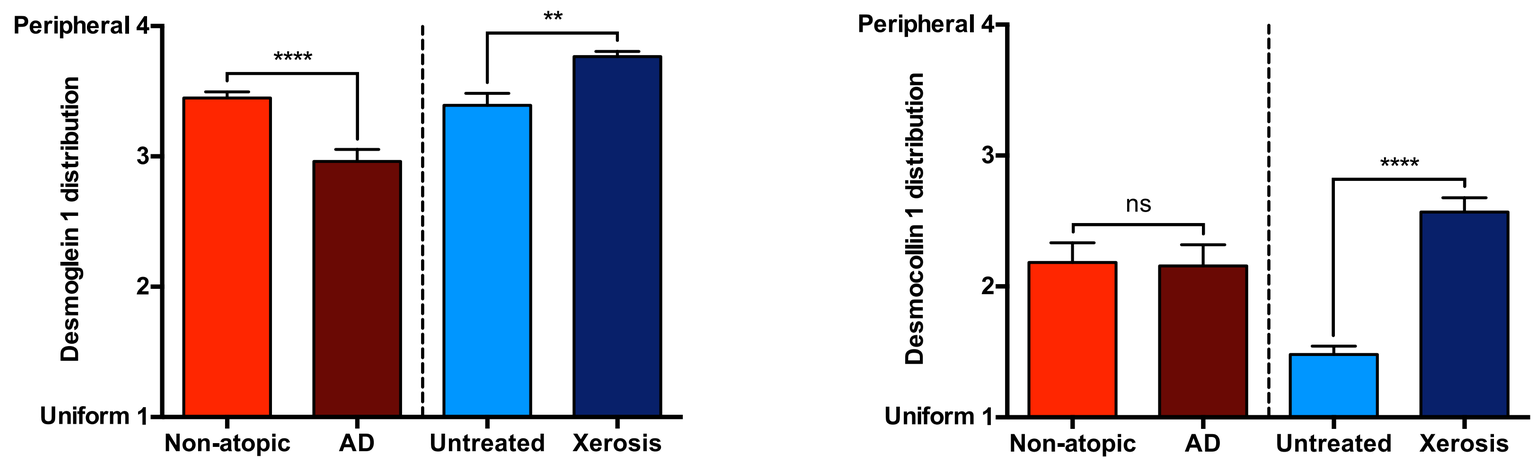

Figure 3: Mean distribution of corneodesmosome adhesion proteins. Desmoglein 1 distribution was more uniform in AD and more peripheral in soap-induced xerosis **(left)**. Desmocollin 1 distribution was unaltered in AD and more peripheral in soap-induced xerosis **(right)**. Significance was identified using non-parametric unpaired (AD) and paired (xerosis) t-tests.

Skin-surface-pH was elevated in both AD and soap-induced xerosis.

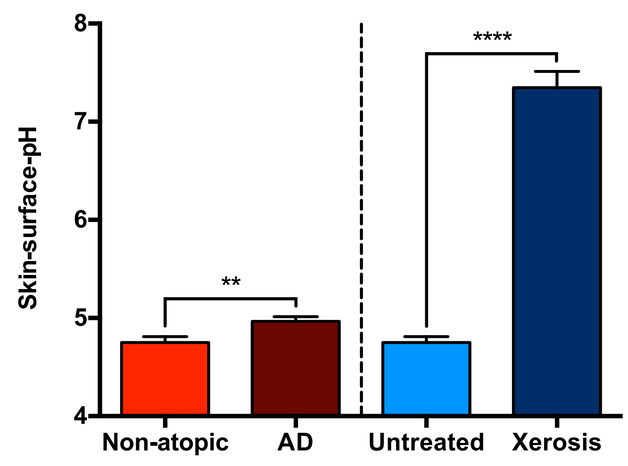

Figure 4: Skin-surface-pH was elevated in both cohorts. The pH of the alkyl carboxylate soap (Imperial Leather) used to induce xerosis is approximately 10[9]. Significance was identified using parametric unpaired t-tests.

Skin barrier function was reduced in both AD and soap-induced xerosis.

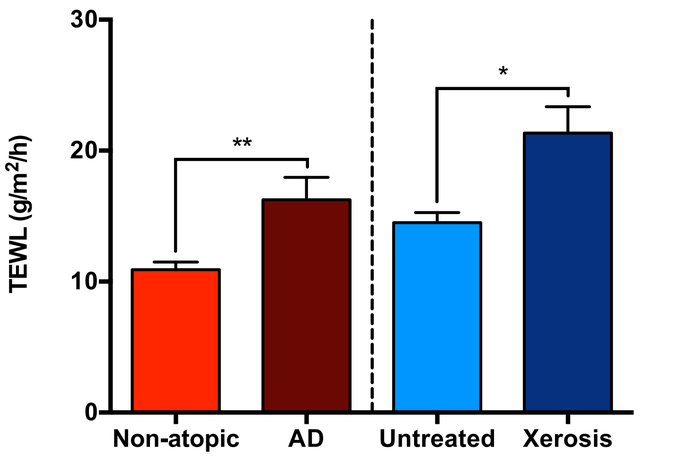

Figure 6: Transepidermal water loss (TEWL), a measure of skin barrier function, was elevated in both AD and soap-induced xerosis, suggesting an impaired barrier in both conditions. Significance was identified using parametric unpaired (AD) and paired (xerosis) t-tests.

Corneocytes had reduced cohesion at the SC surface in soap-induced xerosis.

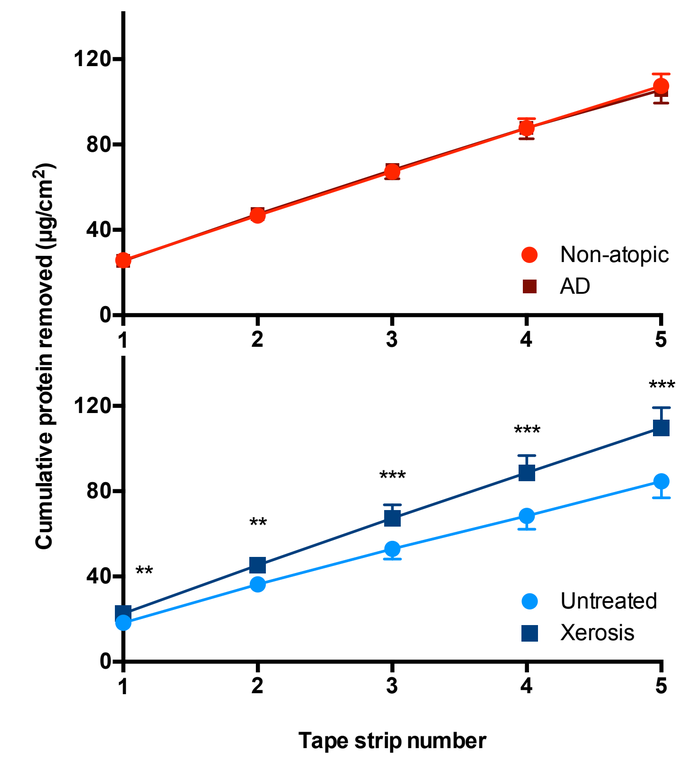

Figure 8: In soap-induced xerosis, an increased amount of SC was removed by 5 consecutive tape strips compared to untreated skin. No such difference was observed in AD. For statistical analysis data was log10 transformed. Significance was identified using a Sidak post-test following a 2-way ANOVA.

Abnormal protease activity was observed in AD and soap-induced xerosis.

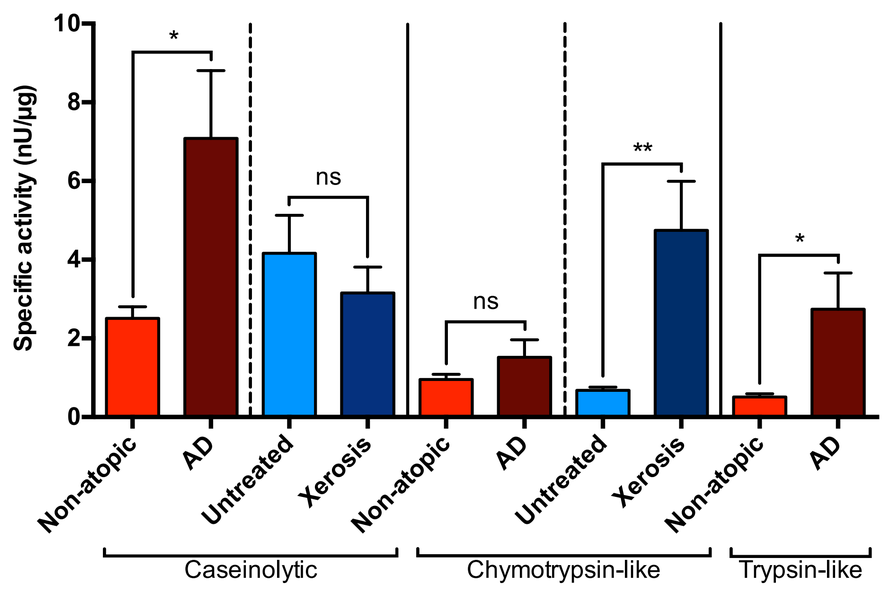

Figure 5: Caseinolytic activity was elevated in AD but there was no significant change in soap-induced xerosis. Chymotrypsin-like activity was elevated in soap-induced xerosis but there was no significant change in AD. Finally, trypsin-like activity was elevated in AD. Significance was identified using non-parametric unpaired (AD) and paired (xerosis) t-tests.

Evidence for premature SC shedding was observed in AD.

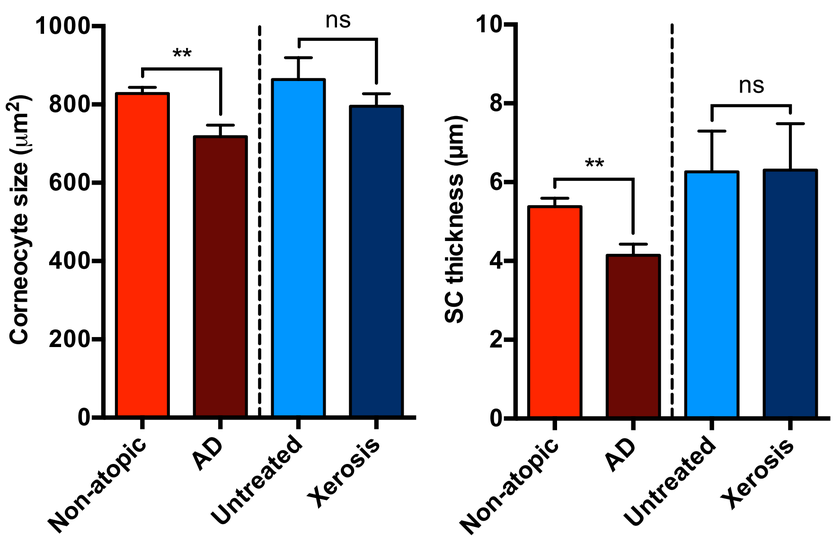

Figure 7: Corneocyte size at the SC surface **(left)** and estimated SC thickness **(right)** were reduced in AD, but there was no significant change in soap-induced xerosis. Significance was identified using parametric unpaired (AD) and paired (xerosis) t-tests.

CONCLUSIONS

- In AD, broadly elevated protease activity leads to increased corneodesmosome degradation, resulting in a defective barrier with SC thinning and immature corneocytes exposed at the SC surface.
- In soap-induced xerosis, elevation of chymotrypsin-like protease activity (but not broad-spectrum/caseinolytic activity) leads to increased degradation of all but the peripheral corneodesmosomes, leaving loosely adhered corneocytes in the upper SC, resulting in a defective barrier exhibiting the flaky characteristics of xerotic skin.