**An investigation of protease activity at non-lesional sites in atopic dermatitis**

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**Background:** Sustained protease hyperactivity within the stratum corneum (SC) is a feature of chronic skin disorders such as Atopic Dermatitis (AD). Animal models have provided a rich source of mechanistic evidence for protease-associated barrier breakdown in AD and related skin diseases, but to date, there is the requirement for further clinical studies in patients. To address this we have developed a simple, non-invasive assay allowing the quantification of 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 desquamatory protease hyperactivity in normal appearing skin at birth. Here, to extend these findings, a cohort study was designed to investigate protease activity in non-lesional adult skin.

**Objective:** To assess surface protease activityin conjunction with the biophysical and molecular properties of the skin barrier in AD patients at non-lesional sites.

**Methods:** Subjects with healthy skin (*n*=20), quiescent AD (*n*=20) and active AD (*n*=68) were recruited for a functional mechanistic study whereby the properties of the skin barrier were assessed using a portfolio of minimally invasive instrumentation, techniques and methodology. Buccal swabs were collected in order to screen participants for the following genetic loci conferring AD risk: *FLG* R501X, 2282del4, R2447X, S3247X, 3702delG; *SPINK5* E420K (LEKTI); and *SCCE* 3’UTR AACC insertion (KLK7).

**Results:** Caseinolytic protease activity at the skin surface was elevated both in subjects with quiescent AD (4.13 ±2.0nU/μg-1 specific activity) and active disease at non-lesional sites (5.69 ±4.7nU/μg-1) compared to healthy skin (2.88 ±1.8nU/μg-1) confirming elevated protease activity in AD. Protease activity correlated with SC hydration (r=-0.53 *p*=<0.0001) and transepidermal water loss (TEWL r=0.62 *p*=<0.0001). Following the removal of the SC surface layers by tape stripping, protease activity correlated with SC lateral lipid chain packing (full width half maximum [FWHM50] of spectral region 1480-1460cm-1) determined by Fourier Transform Infrared Spectroscopy (FWHM50 r=-0.60 *p*=<0.0001). In patients with active disease, no association was observed between surface protease activity and the risk alleles analysed both alone and in combination. Early onset AD and concomitant forms of atopy did not signal increased protease activity at the skin surface.

**Conclusions:** In summary our findings link elevated protease activity to a less orthorhombic SC lipid structure and weakened permeability barrier function in AD. This provides new insight to the pathogenic relationship between elevated protease activity and barrier breakdown in AD at non-lesional sites.