Characterisation of the skin barrier defect in atopic dermatitis using *in vivo* ATR-FTIR molecular spectroscopy

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INTRODUCTION

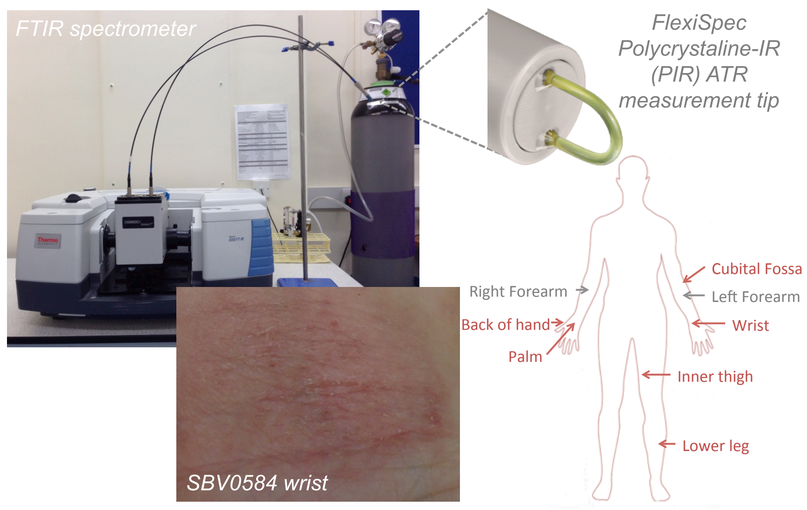
- A defective skin barrier is the underlying cause of atopic dermatitis/ eczema (AD), a common inflammatory skin condition[1]
- This skin barrier defect is characterized by:
  - Abnormal differentiation leading to a defective cornified envelope[1]
  - Decreased levels of natural moisturizing factor (NMF) leading to increased dryness and elevated stratum corneum (SC) pH [2,3]
  - Increased degradatory proteolytic activity [4]
  - Altered composition and conformation of the lipid lamellae, leading to reduced permeability barrier function [5,6]
- Mutations affecting the *FLG* gene, encoding the structural protein filaggrin, confer a skin barrier defect and increase the risk of developing AD (strongest risk factor identified to date).
  - Components of NMF are derived from filaggrin catabolism[7,8]
- Neonates who go on to develop AD already display a skin barrier defect, even before the development of clinical signs[9,10]
- A growing body of evidence suggests that topical emollient therapy to ameliorate the skin barrier defect can prevent the initial onset of AD by 50% and prevent the re-emergence of established AD[11,12]
- Attenuated Total Reflectance (ATR)-Fourier Transform Infrared Spectroscopy (FTIR) is a promising technique for the *in vivo* assessment of skin barrier structure and composition[13,14]

OBJECTIVE: To compare the molecular structure of the skin of AD patients to the skin of healthy controls non-invasively using a fibre-based FTIR device.

METHODS

In a cohort of 56 adult patients with AD, and a control group of 20 volunteers with healthy skin (no skin conditions or atopy), the clinical and biophysical properties of six different skin sites (cubital fossa, volar forearm, wrist, back of hand, palm, and lower leg) were assessed using the techniques listed below (Figure 1 and Table I).

Figure 1: The test sites and equipment



- Erythema, skin surface pH, and skin hydration were determined objectively using a Mexameter, Skin-pH-meter, and Corneometer respectively (C&K, Germany)
- FTIR spectra were collected using a silver halide fibre-optic probe attached to a Nicolet iS50 FTIR spectrometer (Thermo Fisher Scientific Inc), equipped with a cooled MCT detector and purged with dry N_2. 32 scans were collected for each measurement at 4 w/n resolution. Spectral analysis was performed using Omnic 9.0 software (Thermo Electron Corp., Madison, USA).
- The levels of urocanic acid, pyrrolidone carboxylic acid and free amino acids in stratum corneum samples collected on tape-strips (strips 1-3, and 4-6 pooled) were quantified as a measure of NMF levels (method previously described).[8]
- All participants were genotyped for the 5 most common European filaggrin gene mutations using the MenType kit (BioType, Germany).

Statistical analysis was performed in Graphpad Prism 6. Pairs of data were analyzed using a t-test and 3 or more groups were analyzed using ANOVA. The significance level was <0.05. Error bars indicate SEM.

Table I: The study population

Parameter	Healthy	All AD	Mild AD	Moderate AD	Severe AD
Number	20	56	20	26	10
Females	15	35	17	14	4
Mean age	25 (20-45)	27 (18-73)	25 (20-60)	24 (18-53)	40 (19-73)
Severity	NA	24.5 (3.7-14.8)	9.8 (3.7-14.8)	25.0 (15.6-35.5)	52.5 (40.0-66.6)
FLG mutation carriers	1 (5%)	11 (20%)	4 (20%)	5 (19%)	2 (20%)

RESULTS

FTIR spectra of AD patients exhibit prominent changes at several wavelengths associated with NMF components

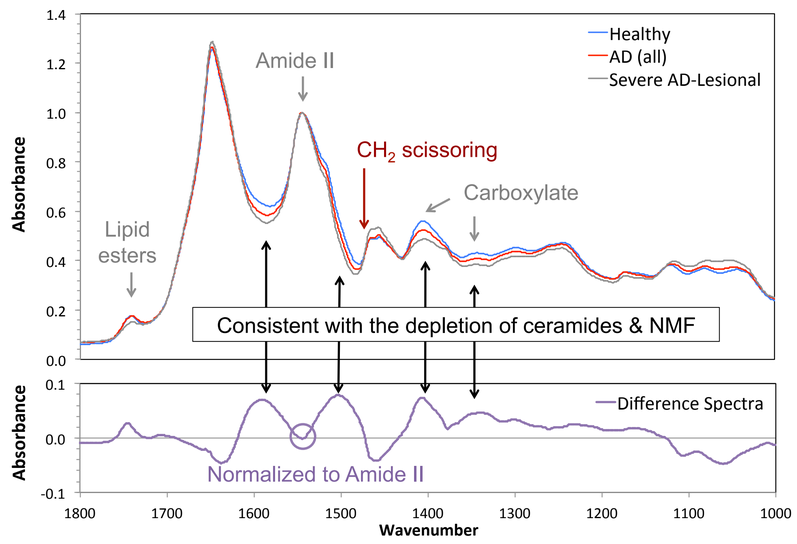


Figure 2: Top: Mean ATR-FTIR absorbance spectra collected at the cubital fossa for healthy particpants (blue line), all AD patients (red line), and severe AD patients with clinical signs at the test sites (grey line). Bottom: Difference spectra (Healthy – all AD).

ATR-FTIR absorbance at 1340 cm^{-1} is a useful biomarker for stratum corneum NMF levels

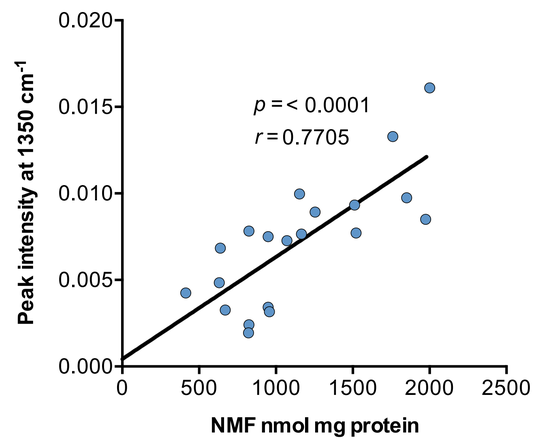


Figure 3: ATR-FTIR absorbance at 1350 cm^{-1} correlates significantly with HPLC determined SC NMF levels in healthy participants on the volar forearm, Pearson's r=0.7705, p<0.0001

ATR-FTIR NMF levels are dependent on anatomical site and skin pathology

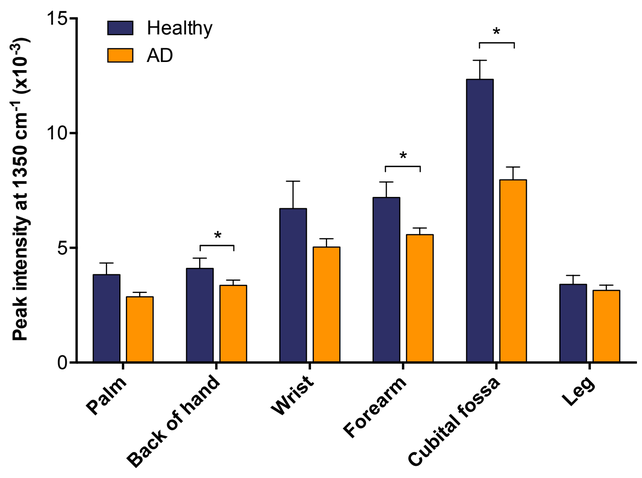


Figure 4: Surface SC NMF levels quantified by ATR-FTIR at different anatomical locations in healthy participants and patients with AD. Asterisks indicate significant differences identified using a t-test.

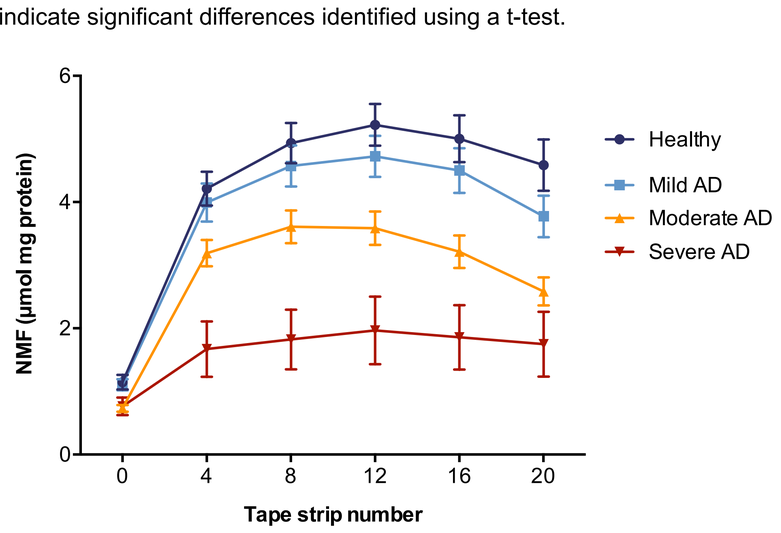


Figure 5: SC NMF levels quantified by ATR-FTIR on the volar forearm before and during tape-stripping in healthy participants and patients with AD. Tape-stripping was performed to quantify NMF levels at deeper levels within the SC. A two-way ANOVA reported significant differences between the groups (post-test results not shown)

ACKNOWLEDGEMENTS

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RESULTS

Surface ATR-FTIR measurements of NMF levels correlate with clinical and biophysical skin properties

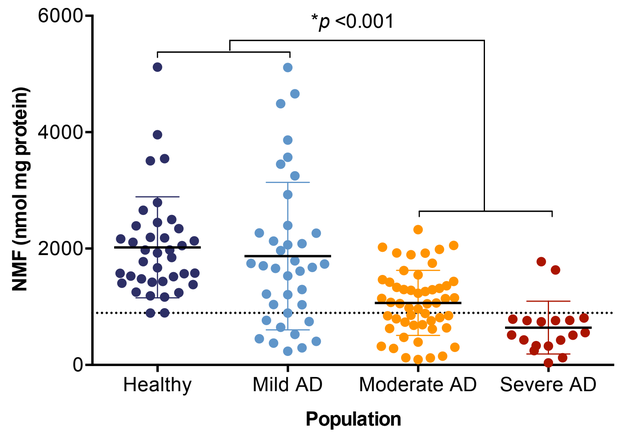

Figure 6: ATR-FTIR determined NMF levels on the cubital fossa stratified by the severity of AD. A one-way ANOVA reported a significant difference between the groups. Asterisks indicate the results of a Tukey post-test. Mean ±SEM displayed.

Parameter	NMF levels Peak intensity 1350 cm^{-1}
SCORAD	r=-0.5529, p<0.0001
Visual Dryness	r=-0.7117, p<0.0001
Erythema (Mexameter)	r=-0.6220, p<0.0001
Hydration (Corneometer)	r=0.5146, p<0.0001
Transepidermal water loss (TEWL)	r=-0.4509, p<0.0001
Skin surface pH	r=-0.3687, p<0.0001

Table II: Correlations between ATR-FTIR determined NMF levels and skin properties

Determination of NMF levels by ATR-FTIR accurately predicts *FLG* gene status

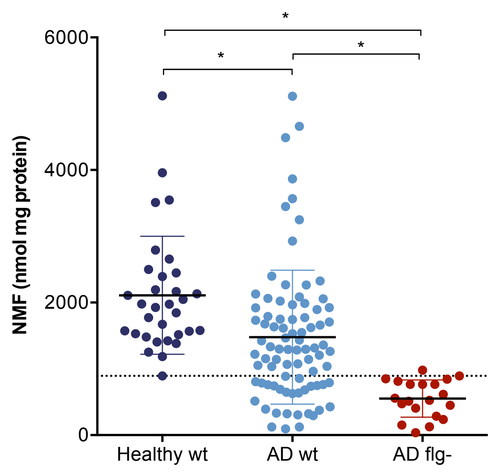

Figure 7: NMF levels on the cubital fossa stratified by *FLG* loss-of-function mutation carriage (het and hom). One-way ANOVA reported a significant difference. Asterisks indicate Tukey post-test results. Mean ±SEM displayed. Receiver operator curve analysis indicated predictive potential (area under the curve 86±0.03%)

AD patients are characterized by low NMF levels and altered lipid structure – two distinct parameters quantifiable using ATR-FTIR

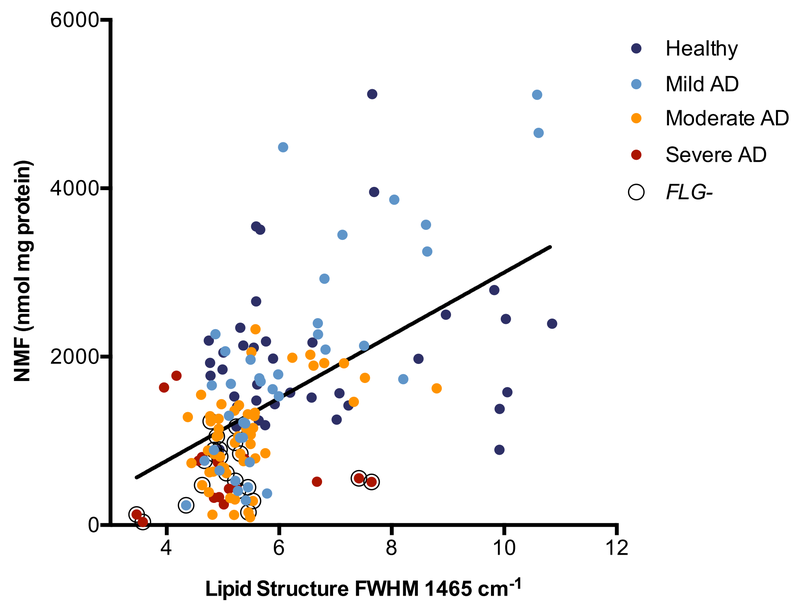

Figure 8: Correlation between stratum corneum NMF levels and and lipid structure. Lipid structure was determined according to a previously published protocol.[13] FWHM, full width at half maximum – a spectral feature associated with lipid membrane lateral chain packing. A highly ordered orthorhombic state (toward FWHM of 12) is associated with optimum permeability barrier function (TEWL)

CONCLUSIONS

ATR-FTIR is a useful technique for the rapid and non-invasive characterisation of the skin barrier defect in AD.

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