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Characterization of skin barrier defects using infrared spectroscopy in patients with atopic dermatitis

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Abstract

Background Atopic dermatitis (AD) is characterized by skin barrier defects that are often measured by biophysical tools that observe the functional properties of the stratum corneum (SC).

Objectives To employ *in vivo* infrared spectroscopy alongside biophysical measurements to analyse changes in the chemical composition of the SC in relation to AD severity.

Methods We conducted an observational cross-sectional cohort study where attenuated total reflection Fourier transform infrared (ATR-FTIR) spectroscopy measurements were collected on the forearm alongside surface pH, capacitance, erythema and transepidermal water loss (TEWL), combined with tape stripping, in a cohort of 75 participants (55 patients with AD stratified by phenotypic severity and 20 healthy controls). Common *FLG* variant alleles were genotyped.

Results Reduced hydration, elevated TEWL and redness were all associated with greater AD severity. Spectral analysis showed a reduction in 1465 cm^{-1} (full width half maximum) and 1340 cm^{-1} peak areas, indicative of less orthorhombic lipid ordering and reduced carboxylate functional groups, which correlated with clinical severity (lipid structure $r=-0.59$, carboxylate peak area $r=-0.50$).

Conclusions ATR-FTIR spectroscopy is a suitable tool for the characterization of structural skin barrier defects in AD and has potential as a clinical tool for directing individual treatment based on chemical structural deficiencies.

What is already known about this topic?

- The skin of patients with atopic dermatitis (AD) is characterized by atypical lipid levels and structure, diminished natural moisturizing factor (NMF) and filaggrin deficiency.
- As a result of these structural alterations, normal skin barrier function is reduced.
- Purpose-built probes or assays are typically used to monitor and quantify biochemical changes, such as pH, moisturization factors, hydration and transepidermal water loss (TEWL), on the epidermal surface as a result of the onset of dermatitis.

What does this study add?

- Characteristics from the mid-infrared spectra collected from participants' skin provided molecular insight into the pathogenesis of AD.
- Lipid- and carboxylate-associated mid-infrared peaks were strongly associated with biophysical measurements, including skin hydration, NMF and TEWL.
- Peaks analysis also indicated a filaggrin deficiency, caused by genetic deposition or inflammatory action.
- Early detection of a genetic predisposition to AD would allow for preventative measures to be undertaken.

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Atopic dermatitis (AD) is a common chronic skin condition characterized by inflammatory lesions and is linked to the development of comorbidities such as food allergies and asthma.[1] Skin affected by AD exhibits multiple physiological abnormalities of the stratum corneum (SC), including altered lipid lamellae composition, reduced filaggrin expression and decreased levels of natural moisturizing factor (NMF).[1,2] These defects negatively affect permeability barrier function, commonly measured by transepidermal water loss (TEWL), skin surface pH and capacitance; however, little pathological insight is gained from these tools alone at the molecular level.[1]

Attenuated total reflection Fourier transform infrared spectroscopy (ATR-FTIR), a form of molecular spectroscopy optimized for the analysis of surfaces, offers potential as a rapid, noninvasive research tool to analyse the molecular make-up of the SC.[3–5] This study focused on the *in vivo* analysis of skin barrier (SB) structure and function in a cohort of adult participants with AD ranging from almost clear to severe and compared them with a healthy control group, using ATR-FTIR spectroscopy as a complementary molecular tool to gold-standard biophysical and biochemical techniques. The aims were to further characterize the structural defects that predispose people to AD, to test the capability of ATR-FTIR as a clinical tool to rapidly assess the specific chemical changes in the SC structure of patients with AD, to identify biomarkers linked to AD risk and to inform the design of future SB interventions.

Materials and Methods

Study design and setting

An observational cohort study was undertaken to compare the structure, function and chemical composition of the SB of patients with AD and healthy controls. A target of 80 participants was set (60 patients with AD and 20 healthy control participants). Recruitment was open to male and female volunteers, who, if they met the specified inclusion criteria, were recruited on a first-come, first-served basis (Table S1; see Supporting Information). Participants underwent a single skin assessment of the right and left volar forearms at the Sheffield Dermatology Research Facility between January and May 2015.

Outcomes

The primary outcomes were the change in molecular composition measured by ATR-FTIR, and the biophysical and biochemical properties of the SC in patients with AD vs. healthy controls using gold-standard assessment tools commonly used in dermatological studies. Secondary outcomes included the modulating effect of *FLG* mutations and emollient use by participants.

Biophysical measurements

All measurements were collected at 19–24 °C and 30–60% relative humidity. Participants were asked not to apply AD-specific topical treatments within 12 h and other topical treatments 1 week prior to measurement collection. Before any measurements were taken, participants exposed their test sites for 20 min. Capacitance, redness and skin surface pH were measured using a corneometer CM825, mexameter MX18 and pH meter PH905 (CK Electronic, Cologne, Germany), respectively, in triplicate. TEWL was measured in duplicate by Aquaflux (Biox Systems, London, UK) as a measure of SB function.

Skin tape stripping

Sites were subjected to skin tape stripping (STS) after all surface measurements were taken. STS involved the repeated application and removal of polyacrylate ester adhesive discs (D-Squame discs; CuDerm, Dallas, TX, USA). To ensure a consistent pressure (225 g cm^{-2}) over the entirety of the disc, a plunger device was applied for 5 s following the application. TEWL and spectroscopy measurements were taken after every 4 sequential tape strips until 20 serial tape strips were removed. The amount of protein removed by tape stripping was quantified using infrared densitometry (SquameScan 850A; Heiland Electronic, Wetzlar, Germany). Equating 100 μm cm^{-2} protein to 1.9±2 μm SC removed by STS allowed the measurement depth to be estimated. The total SC thickness of each participant was estimated through the relationship between mass removed and TEWL, based on Fick's first law, following the methodology detailed elsewhere.[6–9]

Attenuated total reflection Fourier transform infrared spectroscopy

Measurements were taken using a Nicolet™ iS50 spectrometer (Thermo Fisher Scientific, Waltham, MA, USA) fitted with a silver halide probe (Flexispec PIR900; Art Photonics, Berlin, Germany) and a mercury cadmium telluride detector cooled with liquid nitrogen prior to sampling. Nitrogen gas purged the device at 25 psi for the duration of scanning to remove interference from local atmospheric gases. A single measurement was taken on each site, consisting of 32 scans collected for both skin measurements and background at 2 wavenumber resolution. Quantification of peak intensity and location was done using OMNIC 9.0 software (Thermo Electro, Madison, WI, USA). All quantitative peak intensities were calculated relative to the amide II bond of protein at 1550 cm^{-1} to account for different contact pressures. Owing to the penetration depth of ATR-FTIR being approximately 1 μm, STS was undertaken to profile the SC, as in previous studies.[10]

FLG genotyping

Genomic DNA was extracted from buccal swabs using a QIAamp DNA Mini Kit (Qiagen, Hilden, Germany). The five common *FLG* variant alleles were genotyped with a multiplex kit (Mentype; Biotype Diagnostic, Dresden, Germany) and 3730 sequencer (Applied Biosystems, Foster City, CA, USA).

Laboratory analysis of natural moisturizing factor

Discs 1–3 and 4–6 were pooled, extracted in methanol, dried and resuspended in 125 μL of water. Duplicate samples (25 μL) were injected into a high-performance liquid chromatography (HPLC) system (Shimadzu, Kyoto, Japan) equipped with an Aqua C18 column (Phenomenex,

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Table 1 Cohort demographics

	Set analysis					
AD severity	**Clear (healthy; *n*=20)**	**Almost clear (*n*=11)**	**Mild (*n*=23)**	**Moderate (*n*=14)**	**Severe (*n*=7)**	**All (*n*=75)**
Age (years)						
Mean (SD)	24.72 (6.65)	26.71 (12.71)	24.48 (7.78)	24.36 (10.27)	44.69 (24.06)	26.55 (12.05)
Range	20–45	19–60	18–53	19–54	19–73	18–73
Sex (*n*)						
Male	5	3	5	8	4	25
Female	15	8	18	6	3	50
Fitzpatrick skin type, median (range)	2.5 (1–4)	3 (1–4)	3 (1–4)	3 (1–4)	2 (2–4)	3 (1–4)
FLG mutation (*n*)	0	0	6	4	2	12
EASI						
Score boundaries	0	0.1–1	1.1–7	7.1–21	21.1–50	0–50
Mean (range)	–	0.44 (0.20–0.80)	3.40 (1.20–7.00)	11.65 (7.15–18.45)	34.70 (21.10–45.30)	8.69 (0.20–45.30)
Objective SCORAD, mean (range)	–	9.55 (3.70–16.75)	17.71 (7.60–34.50)	32.82 (16.70–62.50)	54.87 (46.50–66.60)	24.61 (3.70–66.60)
Dryness score (right and left mean volar forearms at baseline)						
Mean	0.00	0.10	0.21	0.61	1.08	0.30
Median (range)	0.00 (0.00–0.00)	0.00 (0.00–1.00)	0.00 (0.00–1.50)	0.00 (0.00–1.00)	0.00 (0.00–3.00)	0.00 (0.00–3.00)
Redness score (right and left mean forearms at baseline)						
Mean	0.00	0.00	0.14	0.63	0.81	0.24
Median (range)	0.00 (0.00–0.00)	0.00 (0.00–0.00)	0.00 (0.00–1.00)	0.50 (0.00–0.75)	0.00 (0.75–1.50)	0 (0.00–1.50)
AD lesion (right and left forearms)						
Lesion	0	0	9	18	9	36
No lesion	40	22	37	10	5	114
Emollient use < 24 h prior to skin assessment, *n* (%)	0	4 (36)	7 (30)	8 (57)	5 (71)	24 (32)

AD, atopic dermatitis; EASI, Eczema Area and Severity Index; SCORAD, SCORing Atopic Dermatitis.

Macclesfield, UK) to measure pyrrolidone carboxylic acid (210 nm) and urocanic acid (270 nm). The same sample was used to quantify the free amino acid pool by o-phthalaldehyde derivitization.[11] The sum of each component was normalized to the SC mass removed by STS to give a single, composite measure of NMF (discs 1–6).

Visual scoring

Visual assessments of dryness, redness and overall disease severity [SCORing Atopic Dermatitis (SCORAD) and Eczema Area and Severity Index (EASI)] were recorded for each participant before biophysical measurements were collected (Tables S2, S3; see Supporting Information). EASI score grading boundaries for group assignments are provided in Table 1.

Statistical analysis

Data from all 75 participants were analysed using PRISM 9 (GraphPad Software, La Jolla, CA, USA). Comparisons between healthy participants and AD severity groups were made using a one-way ANOVA and Tukey's post-test. Correlation analysis was reported using Pearson or Spearman *r* values, as indicated. The threshold for statistical significance was $P<0.05$. Results are presented as the mean (SD) unless otherwise stated.

Results

In total, 75 participants consented to take part, and all completed the study. The demographic characteristics of the study group, stratified by AD severity, are shown in Table 1. The mean EASI scores were 3.40, 11.65 and 34.70 for the mild, moderate and severe AD groups, respectively. As the severity of AD increased, forearm skin sites exhibited lower capacitance (a measure of hydration), with a significant 11.9 arbitrary unit (AU) decrease from healthy to severe skin (Figure 1a). TEWL and objective redness increased by 14.3 g m^{-2} h^{-1} and 83.0 AU, respectively, from healthy to severe AD skin (Figure 1b, c). On average, skin surface pH increased from 4.78 in healthy skin to 5.12 in AD skin, but the difference was not statistically significant (Figure 1d). SB integrity was determined by STS in conjunction with TEWL measurements (Figure 1f, g). The depth reached in the SC with STS decreased from 3.78 μm to 2.94 μm, with an increase in AD severity from healthy to severe (Figure 1e). With controlled SC removal, TEWL increased at a greater rate in people with increased AD severity – indicative of weaker SC integrity – with the exception of those in the severe group. This was supported by comparing the area under the curve (AUC), with a significant +94.39 AU mean difference seen between people in the healthy and moderate AD groups ($P=0.004$; Figure 1g).

Average ATR-FTIR spectra from forearm sites for participants in each AD severity group showed broad differences in absorption at the skin subsurface and were used to identify spectral components for further analysis (Figure 2a, b). For example, at 2850, 1465 and 1740 cm^{-1}, the amount and conformational order of SC lipids were analysed. Increased AD severity resulted in a concomitant increase in absorption at 2850 cm^{-1}, highlighting a higher prevalence of total lipids relative to protein in severe AD (0.39 AU) vs. healthy skin (0.22 AU; Figure 3a). A decrease in second-derivative full

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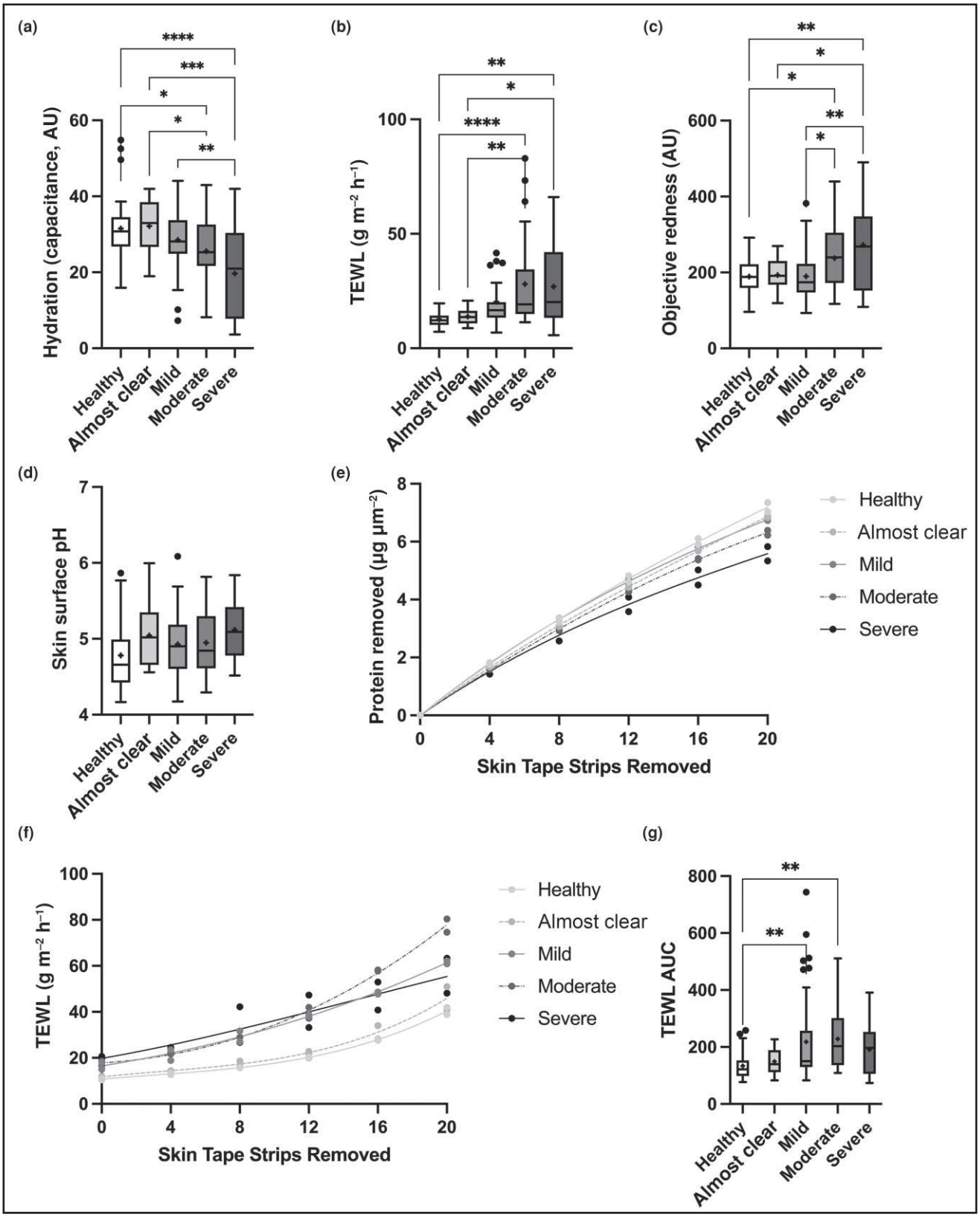

Figure 1 Biophysical measurements of the skin barrier (SB). (a) Skin hydration measured as capacitance; (b) SB function measured as transepidermal water loss (TEWL); (c) objective redness; (d) skin surface pH; (e) cumulative protein removed as a result of skin tape stripping (STS) from 0 to 20 tape strips; (f) TEWL combined with STS as a measure of SB cohesion; (g) SB integrity measured as TEWL area under the curve (AUC) 0–6 μm from the surface of the stratum corneum in response to STS. Box-and-whisker plots depict the minimum, lower quartile, median, upper quartile and maximum values of each group. '+' indicates the mean. Asterisks indicate the results of a one-way ANOVA with Tukey post-test: *$P<0.05$; **$P<0.01$; ***$P<0.001$; ****$P<0.0001$. AU, arbitrary units; ns, not significant.

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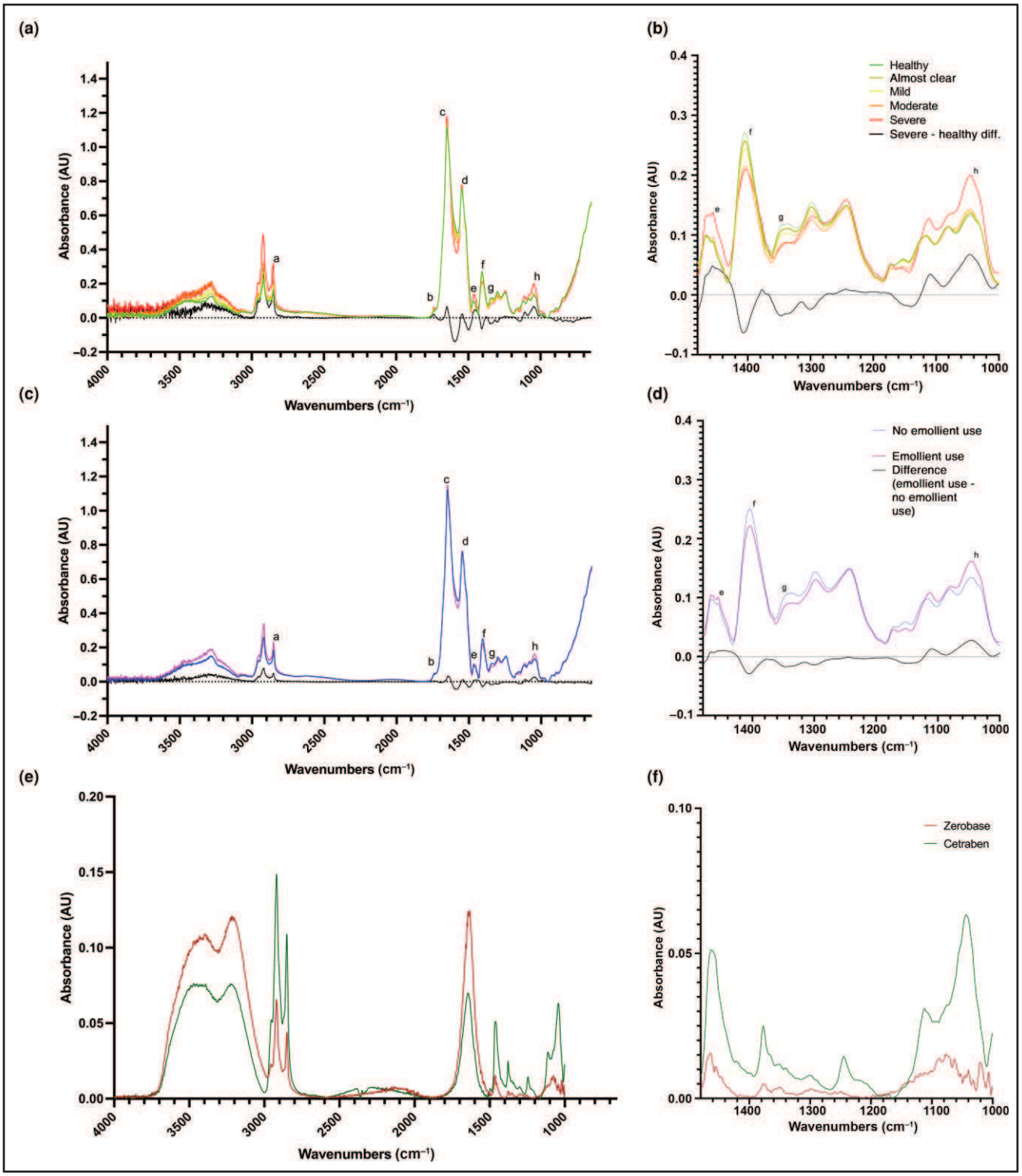

Figure 2 Chemical changes related to disease severity measured by attenuated total reflection Fourier transform infrared spectroscopy. Averaged forearm spectra of all participants within each severity group, after four serial tape strips removed, stratified by (a, b) atopic dermatitis (AD) severity or (c, d) use of emollient within 24 h prior to the study visit. Highlighted peaks correspond to functional groups indicative of (a) total lipid, (b) lipid esters, (c) amide I, (d) amide II, (e) total z-plane lipid, (f, g) carboxylates and (h) polyalcohols. Difference spectra highlight the chemical changes between (a, b) severe AD/healthy skin and (c, d) emollient use. (e, f) Spectra of two commercially available topical emollients are presented. AU, arbitrary units.

width at half maximum (FWHM) peak area at 1465 cm^{-1} (9.84 AU vs. 6.72 AU), combined with a significant shift in absorption frequency at 2850 cm^{-1}, was observed between healthy skin (2850.38 cm^{-1}) and severe AD (2851.06 cm^{-1}), indicative of a more disordered lipid structure with increasing AD severity (Figure 3d, g). As the ATR-FTIR technique is limited to a scanning depth of about 1 μm, a greater SC depth was analysed by combining measurements with STS.

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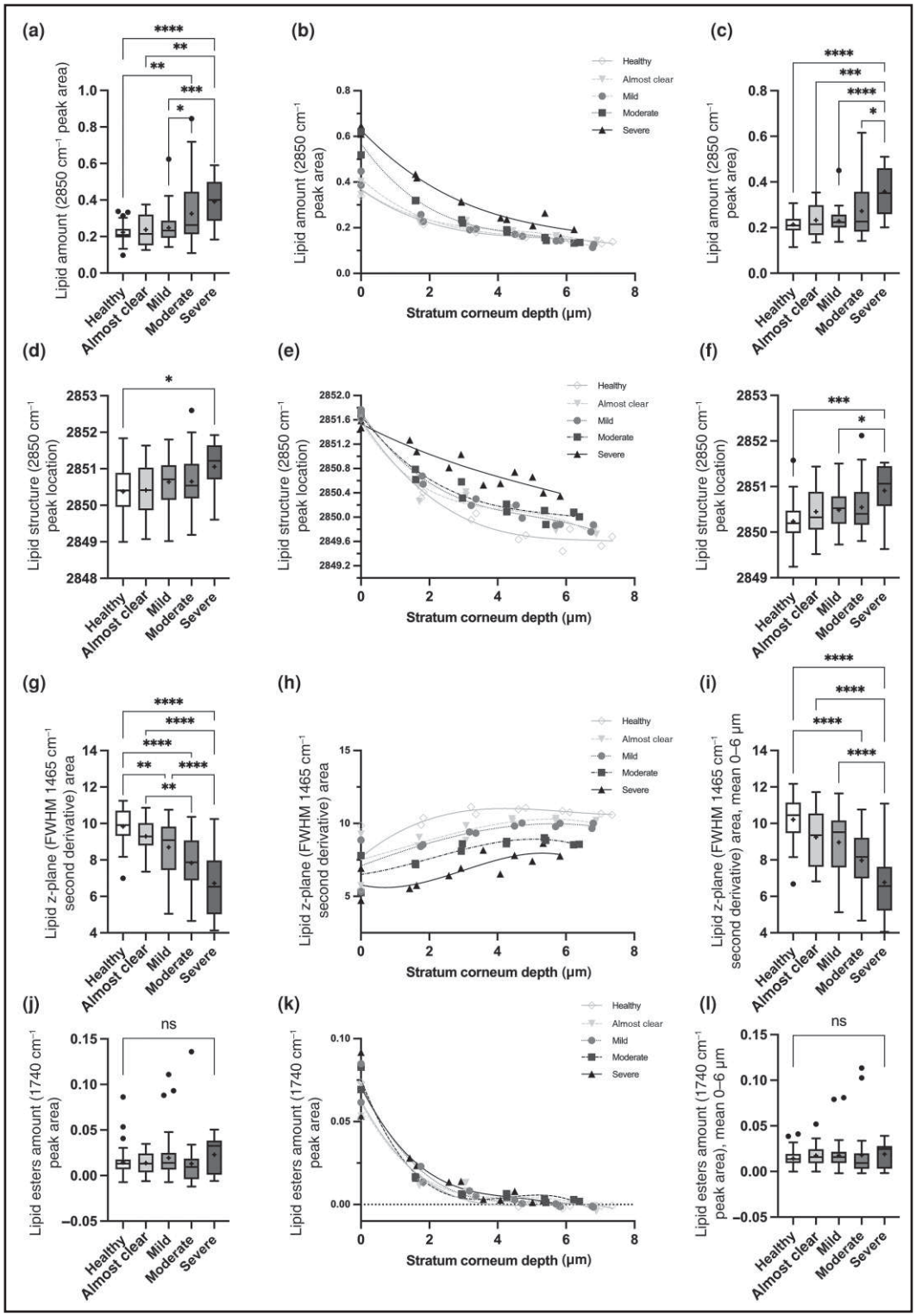

Figure 3 Differences in lipid composition of the skin of people with atopic dermatitis (AD) and healthy controls. Measurement of (a–c) total lipid amount via peak area, (d–f) lamellar lipid structure by lipid peak centre of gravity, (g–i) medial lipid structure (z-plane) through the second derivative area and (j–l) lipid esters through peak area relative to amide II by attenuated total reflection Fourier transform infrared spectroscopy. Spectroscopic parameters were measured after (a, d, g, j) four serial tape strips, (b, e, h, k) repeated until 20 tape strips were attained with (c, f, i, l) the average across 6 μm of stratum corneum presented. Box-and-whisker plots depict the minimum, lower quartile, median, upper quartile and maximum values present in each group. '+' indicates the mean value. Outliers plotted as individual points. Asterisks indicate the results of a one-way ANOVA with Tukey's post-test: *$P<0.05$; **$P<0.01$; ***$P<0.001$; ****$P<0.0001$. FHWM, full width at half maximum; ns, not significant.

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The observed trend of decreased absorbance at 2850 cm^{-1} and 1740cm^{-1} with STS suggested decreasing lipid and lipid ester abundance with increasing depth (Figure 3e, k). Overall, similar increases in lipid amount with a disordered structure were noted between the skin samples of healthy controls and those with severe AD across the full ~6 μm SC analysed (Figure 3c, f, i). However, there was no difference seen in lipid esters between the groups at both the subsurface and throughout the SC (Figure 3j, k, l).

Functional groups linked to SC water content were also assessed via ATR-FTIR peak analysis and broadly showed reduced components of skin hydration associated with AD (Figure 4). The amide I/II peak ratio (an indirect measure of water content) showed significantly decreased hydration in the severe AD group (1.85 AU) compared with healthy participants (1.99 AU) across the full SC analysed (Figure 4c). Peaks at 1410 cm^{-1} and 1340 cm^{-1}, indicative of carboxylate groups common to the constituents of NMF, showed changes with increased disease severity at both the subsurface and throughout the SC (Figure 4g–l). For example, throughout the ~6 μm depth analysed, significant reductions in 1410 cm^{-1} and 1340 cm^{-1} peak areas were observed between healthy and severe AD skin (mean difference 0.049 AU and 0.015 AU, respectively). Spectral NMF derivation at 1340 cm^{-1} was validated by laboratory analysis of NMF from STS across the cohort (Figure S1; see Supporting Information). Here, a significant association was observed between both methods of NMF quantification ($r=0.55$; $P<0.001$), with a reduced 1340 cm^{-1} peak area observed in *FLG* variant allele carriers vs. wild-type carriers, regardless of disease status.

A third of participants applied a topical emollient to the skin within 24 h prior to the assessment.

By stratifying via emollient use, peaks associated with water, lipid and polyalcohol were shown to be elevated as a result of recent emollient application (Figure 2c, d), and the spectra of two common emollients also exhibited prominent peaks associated with water and lipid content (Figure 2e, f), as well as glycerol in the case of Cetraben® (Thornton & Ross, Huddersfield, UK).[12] To ensure the ATR-FTIR results were not significantly affected by emollient use, the analysis was repeated only in those with no recorded emollient use 24 h prior to assessment. As with the full cohort, the lipid amount was elevated and lipid structural alterations, as well as NMF depletion, were seen in patients with severe AD. However, the amount of polyalcohols was significantly lower (0.067 AU) in the severe AD groups with no emollient applied, and skin hydration also showed a greater statistical difference ($P=0.042$ vs. $P=0.001$) between healthy controls and those with severe AD across the SC (Figure S2; see Supporting Information).

Correlation analysis was performed to associate spectroscopic features with biophysical measurements and clinical scoring of disease severity (Figure 5). EASI score was strongly correlated with Z-plane lipid structure ($r=-0.59$) and carboxylate peak area ($r=-0.50$), supporting our prior observation of reduced lipid ordering and low NMF with exacerbated disease severity (Figure 5a, b). Furthermore, z-plane lipid structure is associated with SB function and integrity ($TEWL_{20}$ AUC, $r=-0.57$; Figure 5c), in addition to carboxylate peak area ($r=0.50$; Figure 5d). An increased carboxylate peak area at 1340 cm^{-1} was strongly related to greater skin hydration and reduced skin dryness, as determined spectroscopically ($r=0.70$ and $r=-0.47$, respectively; Figure 5e). SB integrity (TEWL AUC) was inversely associated with spectroscopic skin hydration and lipid z-plane structural changes ($r=-0.57$; Figure 5e).

Discussion

SB breakdown is a key feature of AD. Using a variety of analytical instruments, our results showed that the clinical scoring of disease is associated with the biophysical properties of the skin, with a weakening of skin permeability barrier function and integrity correlating with an escalation of severity. Molecular analysis using *in vivo* ATR-FTIR highlighted concomitant structural differences between clinical groups relating to lipid and NMF components up to an SC depth of 6 μm, highlighting pathological skin changes associated with disease severity that were rapidly determined with minimal invasiveness.

TEWL is used widely in dermatological research as a measure of SB function, and the results obtained in this study (i.e. that increased AD severity results in a higher recorded TEWL reading) are in agreement with other *in vivo* studies that assessed TEWL in participants with conditions that affect the structural integrity of the SC, such as AD.[13–15]

The increase in peak location, derived through centre of gravity (COG), to a higher wavenumber of the ~2850 cm^{-1} lipid peak, with an increase in AD severity, as well as the association shown when correlating this COG with TEWL, is consistent with the results of other *in vitro* and *in vivo* studies,[7,15–19] which have shown that a shift in absorbance is caused by a change in conformation packing order, from an orthogonal towards a more liquid disordered phase. This alteration of lipid packing order is linked to SB function and, therefore, to the severity of AD onset.

Our results showed increased levels of lipid relative to protein present in the skin of participants with severe AD vs. healthy controls, even after the exclusion of participants who had used an emollient within 24 h prior to skin assessment. This may appear to contradict other research in the area, which has linked SC lipid depletion to AD pathogenesis; however, the Raman spectrometer used in the study of Janssens *et al.* scanned at an increased SC depth than in the present study (4–10 μm vs. ≤ 1 μm prior to STS),[20] and it was noted that a greater lipid/protein ratio was present nearer the surface of the skin.[21] With increased SC depth, the difference between relative lipid amounts in various AD severity groups was seen to decrease, which is in agreement with the findings of Janssens *et al.*[20] Ishikawa *et al.* also highlighted that although total absolute ceramide (Cer) levels decreased in patients with AD, the amount of Cer[AS] increased.[22] Shortening of ceramide chain length with an increase in AD severity has previously been identified in other studies, with lipids of chain lengths of < 34 carbon atoms being far more common in people with severe AD than in controls.[15,16,20,22] Previous work has shown that AD treatment with emollients containing ceramides increased the lipid/protein ratio at ~2850 cm^{-1} vs. standard emollient treatment.[23] Thus, a high abundance of short-chain lipids may impact and increase IR absorbance, affecting the lipid/protein ratio, even if absolute lipid amounts present in the SC decrease, as expected, with AD.

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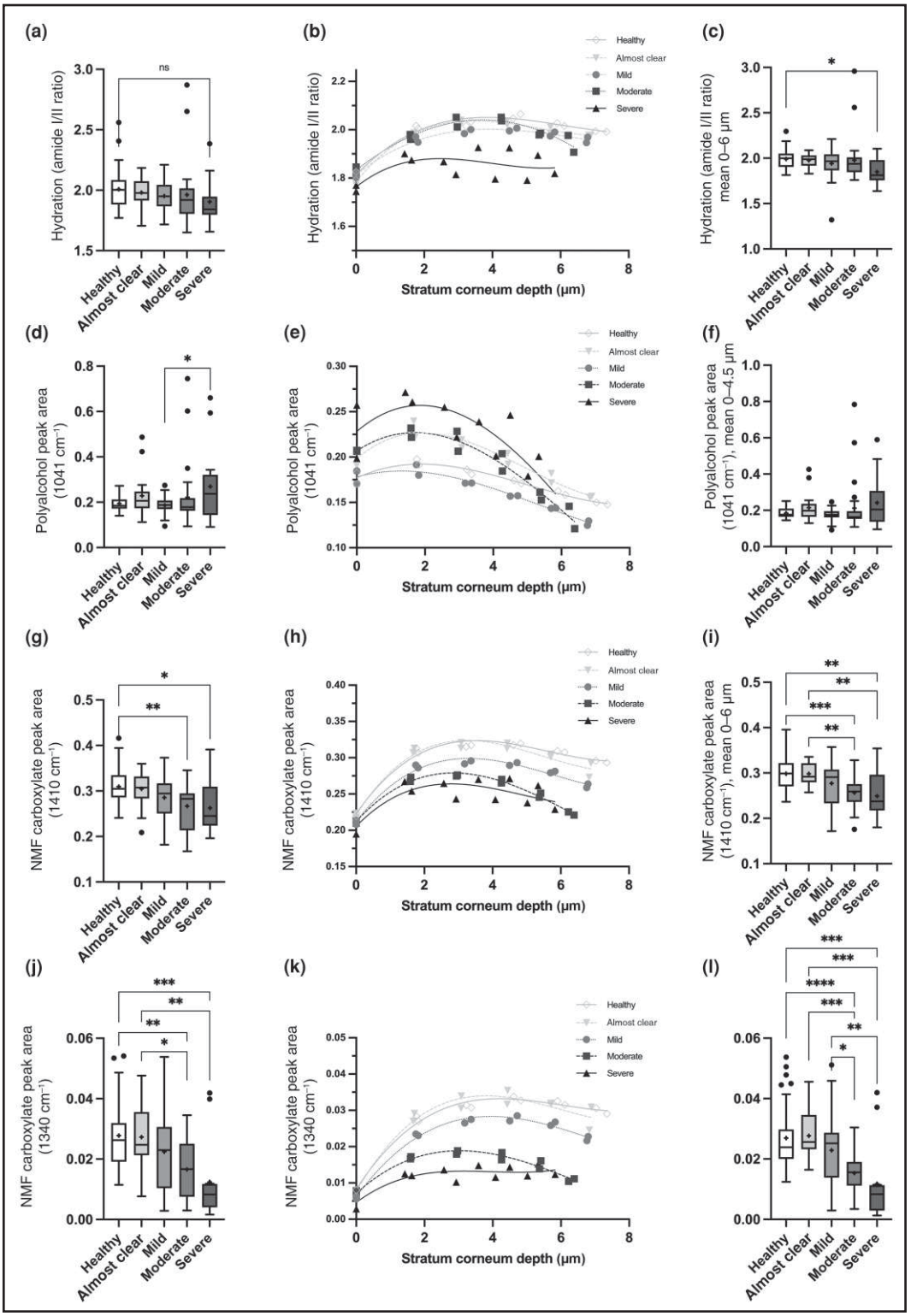

Figure 4 Differences in the nonlipid composition of the skin of participants with atopic dermatitis and healthy controls. Peak area analysis relative to amide II showing (a–c) stratum corneum (SC) hydration, (d–f) polyalcohols such as glycerol and (g–l) carboxylate components of natural moisturizing factor (NMF) by attenuated total reflection Fourier transform infrared spectroscopy. Spectroscopic parameters were measured after (a, d, g, j) four serial tape strips, (b, e, h, k) repeated until a total of 20 serial tape strips was attained with (c, f, i, l) the average across the first 6 μm of SC presented. Box-and-whisker plots depict the minimum, lower quartile, median, upper quartile and maximum values present in each group. '+' indicates the mean value. Outliers are plotted as individual points. Asterisks indicate the results of a one-way ANOVA with Tukey's post-test: $*P<0.05$; $**P<0.01$; $***P<0.001$; $****P<0.0001$). ns, not significant.

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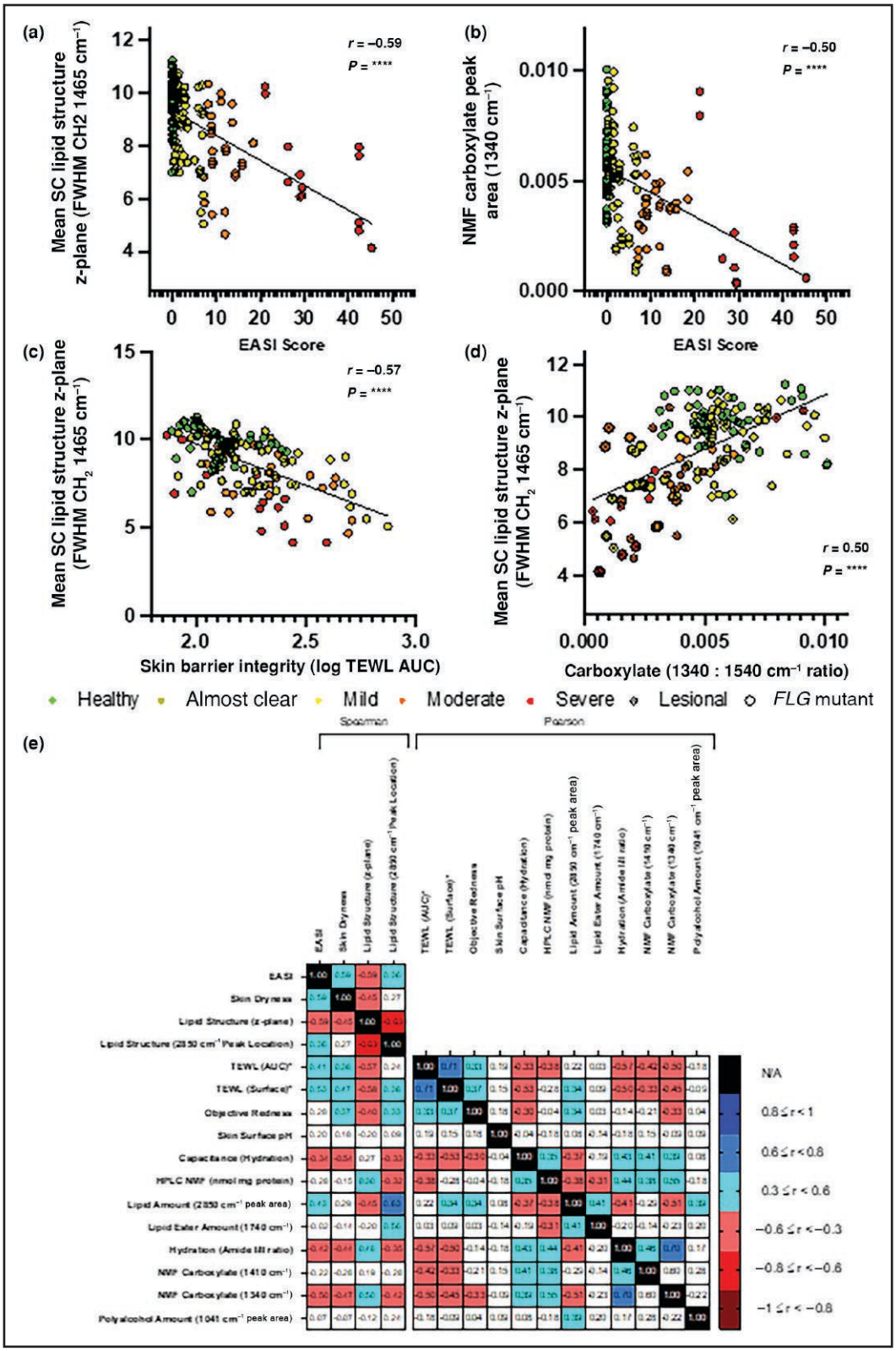

Figure 5 Correlation and component analysis of spectral features and visual skin assessments. (a) Mean stratum corneum (SC) z-plane lipid structure with Eczema Area and Severity Index (EASI) score. (b) Carboxylate amount with EASI score. (c) Mean SC total z-plane lipid with skin barrier integrity. (d) Mean total z-plane lipid structure with carboxylate amount. (e) Correlation matrix of biophysical measurements and spectral features. AUC, area under the curve; FWHM, full width at half maximum; HPLC, high-performance liquid chromatography; N/A, not applicable; NFM, natural moisturizing factor; TEWL, transepidermal water loss. ****$P < 0.0001$.

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Medial lipid structural packing was assessed via the peak centred at 1465 cm^{-1}. Studies have shown that decreased FWHM measurements are caused by the transition from orthorhombic lipid packing to lower-order hexagonal packing, as well as a reduction in lipid chain length, which is also associated with lower SB integrity.[15,18,19] The results seen here concur with those in the literature, with reduced orthorhombic packing seen in severe AD groups vs. less severe AD and healthy skin throughout the SC. A high association was seen when correlating FWHM at 1465 cm^{-1} with TEWL, dryness and NMF abundance, suggesting this peak's potential for use as a marker of SB integrity and SB NMF production defects resulting in the dry, scaly lesions typical of AD.

Dry, cracked skin is a symptom of AD, with increased dryness measured by capacitance associated with a more severe onset.[24] Hydration measurements using ATR-FTIR via the amide I/amide II peak ratio showed reduced water levels in AD throughout the SC, with an overall correlation with capacitance observed.[10,25] The abundance of NMF contributes to the maintenance of SC hydration.

We assessed carboxylate group prevalence in the SC via absorbance at 1340 cm^{-1} and 1410 cm^{-1} wavenumbers, which were validated against direct measurements of NMF via HPLC. This indirectly measured NMF with ATR-FTIR, as carboxylic acid, produced via filaggrin metabolization, is a known component of NMF production.[2,4,20] In agreement with other studies,[4,15] we have shown greater NMF depletion throughout the SC with an increase in AD severity.

Low NMF is associated with AD lesional skin, and *FLG* variant allele carriers suggest, in agreement with other studies, that filaggrin production is important in the regulation of a healthy SB, and gene mutations resulting in a lack of active filaggrin can contribute to the pathogenesis of AD.[14,26–28] Given the role of filaggrin in NMF production and the clustering of filaggrin mutants' skin sites with lesional and increased severity of AD, the close relationship between carboxylates, lipid structure, NMF and skin hydration shown in the correlation analysis validates our hypothesis that filaggrin mutations are linked to skin pathologies typically associated with eczema, such as skin dehydration.[2,4] This clustering of *FLG* mutants with lesional skin sites also shows promise for FTIR-detected downregulation of filaggrin in the SC, resulting in NMF depletion, without the need for sample collection and lengthy laboratory analysis. As a deficiency of filaggrin is caused by genetic predisposition and/or inflammatory action, most strongly exhibited at lesional sites, if used on people prior to the onset of AD, we can qualitatively assess filaggrin production to identify AD risk.[2,29,30]

Correlations with the EASI score suggested that the carboxylate and lipid functional groups assessed contribute to the visual appearance of the skin and may impact the severity of AD. The combined assessment of lipid structure and amount of carboxylate, with the separation of *FLG* mutant and AD lesional skin from controls, further showed the link between filaggrin production and AD. This was validated by the correlations shown, with carboxylate amount and lipid structure inversely associated with both skin hydration and EASI. Uninvolved AD skin clustering close to healthy controls indicated that lipid structure alterations and NMF depletion are most severe within the AD lesions compared with the surrounding skin.

This was an adequately sized study cohort, similar to other *in vivo* observational studies of AD[14,18,20]; to achieve the study's objectives, however, recruitment was biased towards patients with mild AD and lacked those exhibiting more severe symptoms. Volunteers were permitted to use emollients to combat symptoms within 24 h prior to their study visit if required, which impacted the analysis. Affected areas included peaks at 2850 cm^{-1} and 1040 cm^{-1} relating to chemical bonds found in lipids and polyalcohols such as glycerol, which overlap with prominent peaks found in two emollients recommended for AD treatment.[31] It is likely that restricting emollient use further would have discouraged participation and may have further exacerbated recruitment to the severe AD group; we showed similar results after the exclusion of emollient users.

To maximize study participation, participant age was not restricted beyond excluding those < 18 years old, as there are links between SB function, thickness and TEWL with skin growth.[32] Studies have shown a similar elevation in TEWL, indicating reduced barrier function, in children with increasing AD severity, and we believe the structural changes monitored here would also be applicable to SC functional changes in children.[33,34] This study did not involve volunteers with Fitzpatrick skin types from V to VI, who are far less likely to carry *FLG* mutations.[35] We have shown here that *FLG* mutations were not an exclusive driver in filaggrin deficiency, ascertained through NMF abundance, and that lipid structural changes, which can affect all skin types with AD, are relevant to filaggrin production.

We have provided molecular characterization of the lipid defect in the SC of participants with AD vs. controls through ATR-FTIR. Associations between spectral features and surface biophysical measurements, such as carboxylate and amide I–II peak relationships correlating with the laboratory-assessed NMF volume from the skin surface, indicate the potential of ATR-FTIR as a multifunctional tool capable of collecting measurements typically recorded by purpose-built devices and/or time-consuming and costly laboratory assays. Through the combined assessment of peaks associated with lipid structure and carboxylate amount, filaggrin deficiency – through either genetic predisposition (*FLG* mutations) through inflammatory action, which is related to the presence of NMF in the SC, skin hydration and overall severity of AD – has been identified with ATR-FTIR. This highlights the potential use of ATR-FTIR as a clinical bedside tool that could rapidly identify biomarkers important in the progression of AD and help direct individual treatment on a case-by-case basis. Identification of biomarkers such as filaggrin deficiency in children could help identify those at risk of developing AD so that preventative measures may be enacted.

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Conflicts of interest

The authors declare no conflicts of interest.

Data availability

The data underlying this article will be shared upon reasonable request to the corresponding author.

Ethics statement

This study was approved by the National Research Ethics Service (NRES) Committee East Midlands – Derby, under the study reference 04/MRE04/70. Informed consent was obtained prior to participation, and the study performed in accordance with the Declaration of Helsinki 1964 and its later amendments.

Supporting Information

Additional Supporting Information may be found in the online version of this article at the publisher's website.

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*SOTYKTU was studied in two global, Phase 3, randomised, multi-arm clinical studies: POETYK PSO-1 and PSO-2. **PASI 75 and sPGA 0/1 vs. placebo at Week 16 were co-primary endpoints**. PASI 75 was defined as ≥75% reduction from baseline in the Psoriasis Area and Severity Index. sPGA was defined as sPGA score of 0 or 1 with ≥2-point improvement from baseline. N numbers: PSO-1: SOTYKTU (n=332); apremilast (n=168), placebo (n=166); PSO-2: SOTYKTU (n=511); apremilast (n=254), placebo (n=255). SOTYKTU delivered superior PASI 75 response rates vs placebo (PSO-1: 58.4% vs. 12.7%, p<0.0001; PSO-2: 53.0% vs. 9.4%, p<0.0001) at Week 16, and superior results achieving clear or almost clear skin (sPGA 0/1) vs. placebo (PSO-1: 53.6% vs. 7.2%, p<0.0001; PSO-2: 49.5% vs. 8.6%, p<0.0001) at Week 16 (co-primary endpoints).[2,3]

†Via enzyme inhibition, enzyme induction, or transporter inhibition.[1]

Abbreviations: AE, adverse event; DDI, drug–drug interaction; PASI, Psoriasis Area and Severity Index; sPGA, static Physician's Global Assessment; TYK2, tyrosine kinase 2.

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Bristol Myers Squibb™

Job code: 1787-GB-2300026 Date of preparation: September 2023

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Great Britain

Consult Summary of Product Characteristics (SmPC) before prescribing. **This medicinal product is subject to additional monitoring. This will allow quick identification of new safety information.**

PRESENTATION: Film-coated tablet containing 6 mg of deucravacitinib.

INDICATION: Treatment of moderate to severe plaque psoriasis in adults who are candidates for systemic therapy.

DOSAGE AND ADMINISTRATION: Treatment should be initiated under the guidance and supervision of a physician experienced in the diagnosis and treatment of psoriasis. Posology: 6 mg orally once daily. If a patient shows no evidence of therapeutic benefit after 24 weeks, treatment discontinuation should be considered. The patient's response to treatment should be evaluated on a regular basis. Special populations: *Elderly:* No dose adjustment is required in elderly patients aged 65 years and older. Clinical experience in patients ≥ 75 years is very limited and deucravacitinib should be used with caution in this group of patients. *Renal Impairment:* No dose adjustment is required in patients with renal impairment, including end stage renal disease (ESRD) patients on dialysis. *Hepatic impairment:* No dose adjustment is required in patients with mild or moderate hepatic impairment. Deucravacitinib is not recommended to be used in patients with severe hepatic impairment. *Paediatric population:* The safety and efficacy of deucravacitinib in children and adolescents below the age of 18 years have not yet been established. No data are available. Method of administration: For oral use. Tablets can be taken with or without food. Tablets should be swallowed whole and should not be crushed, cut, or chewed.

CONTRAINDICATIONS: Hypersensitivity to the active substance or to any of the excipients (see SmPC). Clinically important active infections (e.g. active tuberculosis).

WARNINGS AND PRECAUTIONS: Infections: Treatment should not be initiated in patients with any clinically important active infection until the infection resolves or is adequately treated. Caution should be exercised when considering the use in patients with a chronic infection or a history of recurrent infection. Patients treated with deucravacitinib should be instructed to seek medical advice if signs or symptoms suggestive of an infection occur. If a patient develops a clinically important infection or is not responding to standard therapy, monitor carefully and deucravacitinib should not be given until the infection resolves. Pre-treatment evaluation for tuberculosis (TB): Prior to initiating treatment with deucravacitinib, patients should be evaluated for TB infection. Deucravacitinib should not be given to patients with active TB. Treatment of latent TB should be initiated prior to administering deucravacitinib. Anti-TB therapy should be considered prior to initiation of deucravacitinib in patients with a past history of latent or active TB in whom an adequate course of treatment cannot be confirmed. Patients receiving deucravacitinib should be monitored for signs and symptoms of active TB. Malignancies*: Malignancies, including lymphomas and non-melanoma skin cancer (NMSC), were observed in clinical studies with deucravacitinib. Limited clinical data are available to assess the potential relationship of exposure to deucravacitinib and the development of malignancies. Long-term safety evaluations are ongoing. The risks and benefits of deucravacitinib treatment should be considered prior to initiating patients**. Major adverse cardiovascular events (MACE), deep venous thrombosis (DVT) and pulmonary embolism (PE)*: An increased risk was not observed in clinical trials with deucravacitinib. Long-term safety evaluations are ongoing. The risks and benefits of deucravacitinib treatment should be considered prior to initiating patients**. Immunisations: Consider completion of all age-appropriate immunisations according to current immunisation guidelines prior to initiating therapy. Use of live vaccines in patients being treated with deucravacitinib should be avoided. Excipients: Contains lactose. Patients with rare hereditary problems of galactose intolerance, total lactase deficiency or glucose galactose malabsorption should not take this medicinal product. Contains less than 1 mmol of sodium (23 mg) per tablet, essentially 'sodium-free.' *serious. **It is not known whether tyrosine kinase 2 (TYK2) inhibition may be associated with the adverse reactions of Janus Kinase (JAK) inhibition. In a large randomised active-controlled study of a JAK inhibitor in rheumatoid arthritis (RA) patients 50 years and older with at least one additional cardiovascular risk factor, a higher rate of malignancies (particularly lung cancer, lymphoma and NMSC), a higher rate of MACE (defined as cardiovascular death, non-fatal myocardial infarction and non-fatal stroke), and a dose dependent higher rate of venous thromboembolism (including DVT and PE) were observed with a JAK inhibitor compared to TNF inhibitors.

INTERACTIONS: Deucravacitinib does not have any known clinically relevant drug interactions. Refer to SmPC for full details.

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UNDESIRABLE EFFECTS: The most commonly reported adverse reaction is upper respiratory infections (18.9%), most frequently nasopharyngitis. The longer-term safety profile of deucravacitinib was similar and consistent with previous experience. Very common (≥ 1/10): Upper respiratory infections*** (including nasopharyngitis, upper respiratory tract infection, viral upper respiratory tract infection, pharyngitis, sinusitis, acute sinusitis, rhinitis, tonsillitis, peritonsillar abscess, laryngitis, tracheitis, and rhinotracheitis). Common (≥ 1/100 to < 1/10): Herpes simplex infections*** (including oral herpes, herpes simplex, genital herpes, and herpes viral infection), Oral ulcers (including aphthous ulcer, mouth ulceration, tongue ulceration, and stomatitis), Acneiform rash (including acne, dermatitis acneiform, rash, rosacea, pustule, rash pustular, and papule), Folliculitis and Blood creatine phosphokinase increased. Uncommon (≥ 1/1,000 to < 1/100): Herpes zoster***. Refer to SmPC for full details on adverse reactions.

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DATE OF PREPARATION: May 2023

ADDITIONAL INFORMATION AVAILABLE ON REQUEST

Approval code: 1787-GB-2300080

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Northern Ireland / Ireland

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***serious adverse drug reaction

LEGAL CATEGORY: POM

MARKETING AUTHORISATION NUMBER and BASIC NHS PRICE: EU/1/23/1718/006: Carton of 28 film-coated tablets 6 mg NHS price: £690.00.

MARKETING AUTHORISATION HOLDER: Bristol-Myers Squibb Pharma EEIG, Plaza 254, Blanchardstown Corporate Park 2, Dublin 15, D15 T867, Ireland.

FOR FURTHER INFORMATION CONTACT:

medical.information@bms.com or 0800 731 1736 (Northern Ireland) / 1 800 749 749 (Ireland).

DATE OF PREPARATION: June 2023

ADDITIONAL INFORMATION AVAILABLE ON REQUEST

Approval code: 1787-IE-2300001

Adverse events should be reported. Reporting forms and information can be found at: Northern Ireland – www.mhra.gov.uk/yellowcard or search for MHRA Yellow Card in the Google Play or Apple App Store; Ireland – via HPRA Pharmacovigilance at www.hpra.ie Adverse events should also be reported to Bristol-Myers Squibb via medical.information@bms.com or 0800 731 1736 (Northern Ireland); 1 800 749 749 (Ireland).