

ORIGINAL ARTICLE

Concentration of filaggrin monomers, its metabolites and corneocyte surface texture in individuals with a history of atopic dermatitis and controls

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Abstract

Background Atopic dermatitis (AD) is characterized by skin barrier dysfunction. Notably, a high number of nano-scale protrusions on the surface of corneocytes, which can be expressed by the Dermal Texture Index (DTI), were recently associated with paediatric AD, loss-of-function mutations in filaggrin gene (*FLG*) and reduced levels of natural moisturizing factors (NMF). No study has so far examined the association between these parameters and monomeric filaggrin levels in adults.

Objective To determine DTI, monomeric filaggrin and NMF in healthy controls and a group of patients with controlled dermatitis.

Methods A total of 67 adults (20 healthy controls and 47 dermatitis patients) were included. In the patient population, a personal history of AD was diagnosed by the U.K. Working Party's Diagnostic Criteria. All participants were tested for *FLG* mutations (R501X, 2282del4, R2447X). Transepidermal water loss, monomeric filaggrin, DTI and NMF were measured.

Results In the patient population, 78.7% (37/47) had a history of AD and 59.5% (28/47) had *FLG* mutations. Patients had significantly higher levels of DTI and significantly lower levels of monomeric filaggrin and NMF compared to the 20 healthy controls. Among patients, reduced level of monomeric filaggrin and NMF correlated with the presence of *FLG* mutations and clinical phenotypes such as xerosis, palmar hyperlinearity and AD. Among healthy controls, DTI was significantly higher in the oldest age group compared to the two younger age groups.

Conclusion A significant difference in DTI, monomeric filaggrin and NMF levels was found when comparing dermatitis patients with healthy controls. These findings suggest that even mild dermatitis or non-visible inflammation has a significant and negative effect on the skin barrier as inflammation is known to reduce filaggrin levels. DTI was significantly increased in aged individuals in the healthy control group, suggesting a gradual change in corneocyte morphology with age.

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Conflicts of interest

The authors have no conflict of interest to declare.

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Introduction

Human stratum corneum (SC) consists of multiple layers of protein-enriched, flattened and smooth corneocytes in a matrix of hydrophobic lipids, resembling ‘bricks and mortar’.¹ In case of skin barrier impairment and atopic dermatitis (AD), nano-scale protrusions on the corneocyte surface may appear (Fig. 1).^{2,3} These protrusions have in the past been described as ‘villus-like projections’ or ‘circular nano-objects’, but neither their function nor their composition has been identified.

Recently, a novel method was developed to accurately count these corneocyte protrusions, and the Dermal Texture Index (DTI) was established to enable comparative studies³. Importantly, DTI was higher in both non-lesional and lesional skin from paediatric AD patients and correlated with the presence of common loss-of-function mutations in filaggrin gene (*FLG*).^{3,4} Furthermore, a strong inverse correlation between DTI and the degradation products of filaggrin, natural moisturizing factors (NMF), was observed.⁴

As AD, and other forms of dermatitis, is characterized by primary or secondary skin barrier impairment, there is a need for additional studies that examine the difference in various novel biomarkers between dermatitis patients and controls. For example, cytokines that are active in innate and acquired immune response of AD down-regulate *FLG* expression and reduce filaggrin monomer levels.⁵ In this study, we determined DTI as well as the quantity of epidermal monomeric filaggrin and NMF in healthy controls and dermatitis patients with controlled disease.⁶

Material and methods

Study population

A total of 67 Caucasian individuals aged 18–68 years (20 healthy controls and 47 dermatitis patients with controlled disease) participated in the study between October 2011 and March 2012. Healthy controls were recruited by online advertisement. The patient population was recruited by reviewing medical charts of patients with dermatitis at the Department of Dermatology and Allergy, Herlev and Gentofte Hospital, Denmark. Participants were invited in connection with a previous study by Bandier *et al.*⁶ That study demonstrated a dose-dependent correlation between epidermal monomeric filaggrin levels and *FLG* mutations and included both homozygous and heterozygous carriers

Prior presentations: The content has not been published previously and is not otherwise submitted for publication.

[†]This work is dedicated to our dear friend and colleague, Niels H. H. Heegaard, who suddenly and sadly passed away.

[‡]Equal responsibilities as first authors.

of common *FLG* mutations.⁶ Patients were ineligible for participation if they had active or widespread dermatitis (mild eczema was accepted), other chronic inflammatory diseases besides eczema (i.e. rheumatoid arthritis, Crohn’s disease, ulcerative colitis, systemic lupus erythematosus or psoriasis), or underwent ultraviolet (UV) irradiation 3 weeks before study start, had used topical corticosteroids within 2 weeks or used systemic immunosuppressants. The healthy controls had no current or past history of dermatitis; otherwise, the same exclusion criteria applied for the healthy controls as for the patient population. Blood samples were collected from the healthy controls for *FLG* genotyping. The participants were instructed not to use moisturizers on the day of measurements and skin sampling.

Atopic dermatitis and filaggrin genotype

In the patient population, a personal history of AD was diagnosed by the U.K. Working Party’s Diagnostic Criteria and represented ‘AD ever’.⁷ Current AD was not assessed. All participants were tested for three of the most common *FLG* loss-of-function mutations among Northern European populations (R501X, 2282del4 and R2447X) by multiplex PCR analysis.^{8,9}

Clinical assessment of the participants and history of atopic diseases and symptoms

J. Bandier assessed and registered clinical skin features such as xerosis, palmar hyperlinearity and keratosis pilaris. Furthermore, participants were asked whether they had a history of asthma or hay fever, whether they ever had experienced fissures on their hands and feet and questions related to the diagnosis of AD according to the U.K. Working Party’s Diagnostic Criteria.⁷

Transepidermal water loss

TEWL was measured with the MPA5 (Multiprobe Adaptor system, Courage and Khazaka Electronics GmbH, CK Electronics, UK) at the inner surface of the left upper arm (opposite side of where the punch biopsy for epidermal filaggrin monomer quantification was taken). TEWL [(g/m²)/h] was measured for 30 s, and the average of the 10 last seconds of steady state was calculated and used in the statistical analyses.

Corneocyte surface morphology and DTI

Corneocytes were sampled by a tape-stripping technique where round, adhesive tapes (3.8 cm², D-Squame, CuDerm, Dallas, TX, USA) were attached to healthy or non-lesional skin on the volar forearm. Tapes were applied for 5s by the use of a pressure applicator (D500 – D-Squame Pressure Instrument, CuDerm, Dallas, TX, USA),¹⁰ gently removed by tweezers and stored in a

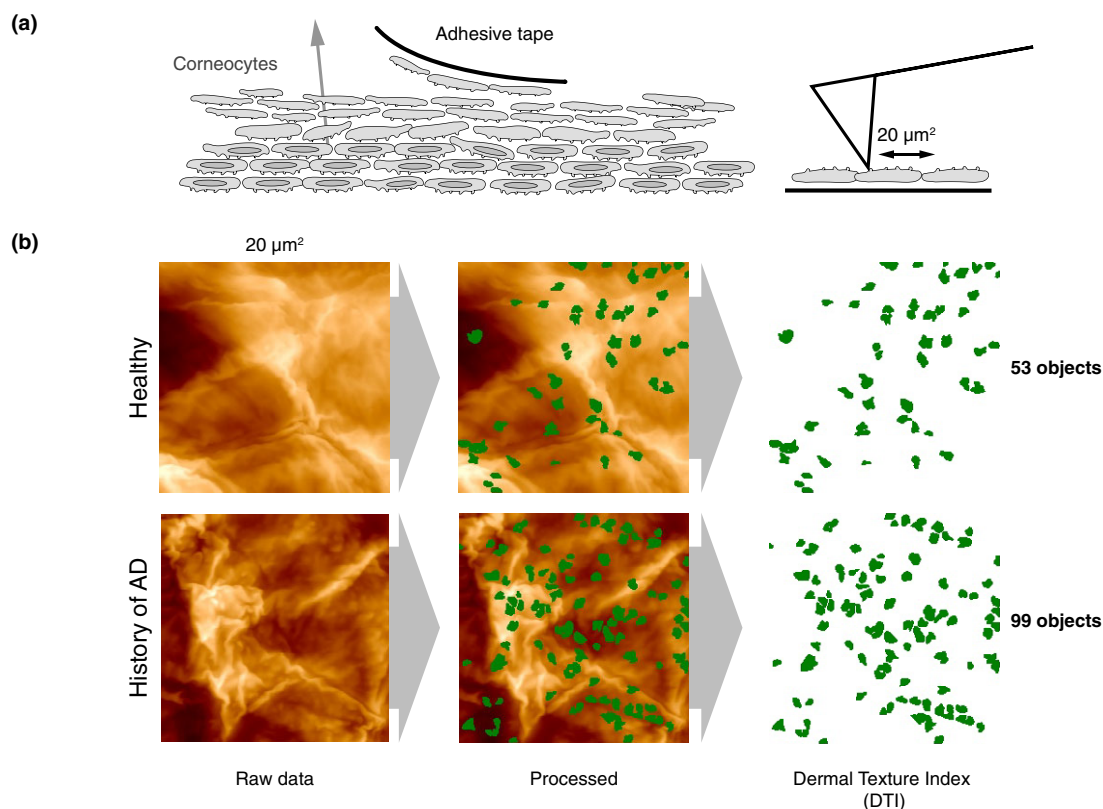


Figure 1 Nanostructure counting. (a) Superficial corneocytes are obtained by tape stripping. The basal (bottom) face of corneocytes adhering to the tape is imaged by atomic force microscopy (AFM). (b) Arbitrarily addressed areas of 20 µm² are scanned and analysed by computer vision to count circular structures (green spots). The number of objects per area yields the Dermal Texture Index (DTI). DTI is defined as mean value of 10 images. AD, atopic dermatitis.

closed vial until analysis. The same skin location was tape-stripped five consecutive times, and the fourth tape was analysed by atomic force microscopy (AFM) as described in details elsewhere.¹¹ Briefly, a Multimode AFM equipped with the Nanoscope III controller and software version 5.30sr3 (Digital Instruments, Santa Barbara, CA, USA) and silicon nitride tips on V-shaped gold-coated cantilevers were used (0.01 N/m, MLCT, VEECO, Mannheim, Germany). The topographic cell surface data were analysed using the nAnostic method with custom-built proprietary algorithms (Serend-ip GmbH, Munster, Germany).¹² Computer vision was used to evaluate each nanostructure morphometrically and to filter them by size and shape. For each sample, 10 randomly selected images of 20 µm² were assessed, and the mean value of the identified objects in these 10 images gave the final DTI.

Determination of filaggrin degradation products in stratum corneum

Corneocytes were collected by the same tape-stripping technique as described previously to determine the amount of filaggrin degradation products, and the analyses were also

performed on the fourth consecutive tape.¹³ Briefly, NMF components, histidine (His), 2-pyrrolidone-5-carboxylic acid (PCA) and urocanic acid (UCA) (trans- and cis-isomer), were extracted from each tape by adding 500 µl 25% (w/w) ammonia solution. After 2 h of continuous shaking (IKA-Vibrax Model 2200, IKA-works Inc, Wilmington, NC, USA), the extracts were evaporated to dryness at 60 °C (Eppendorf Concentrator 5301, Eppendorf AG, Hamburg, Germany) and the residue was dissolved in 500 µl Millipore water and analysed by HPLC. To compensate for the variable amount of stratum corneum protein on the tape strips, the total amount of protein was determined. Due to incomplete extraction of proteins by ammonia, a second extraction with 0.1 M KOH was performed. Proteins in both extractions were measured by a Pierce Micro BCA protein assay kit (Thermo Fischer Scientific, Rockford, IL, USA), added together, and the levels of NMF were expressed as mmol/g total protein.¹³

Quantification of epidermal monomeric filaggrin in the skin

Epidermal monomeric filaggrin was quantified in 4 mm skin biopsies taken from the inner surface of the upper arm.⁶ Biopsies

were stored in Eppendorf vials containing storage buffer (10 mM potassium phosphate, 2 mM Na₂EDTA, pH 7) and placed at –80 °C until analysis. The epidermis was peeled off after incubation at 56 °C for 10 min, and then, the epidermal protein was extracted by the use of mincing, extraction buffer, grinding, sonication, centrifugation, delipidation and dialysis. Quantification of the monomeric filaggrin level was performed by an in-house enzyme-linked immunosorbent assay (ELISA) as previously described.⁶ The level of monomeric filaggrin was expressed as AU/mg epidermis.

Statistics

To determine whether the data were normally distributed, we used the Shapiro–Wilk test. The DTI and levels of monomeric filaggrin and NMF are presented as median values (25th/75th percentiles). In the patient population, NMF and DTI data were log-transformed to obtain normal distribution. The independent sample *t*-test was used to compare two groups, while one-way ANOVA followed by the Tukey *post hoc* test was used for multiple comparisons. For monomeric filaggrin levels, the Mann–Whitney *U*-test and Kruskal–Wallis test with pairwise Mann–Whitney *U*-test *post hoc* tests were used for comparisons of two groups and multiple groups, respectively. In the healthy controls, data on DTI, monomeric filaggrin and NMF were log-transformed to obtain normal distribution and the independent samples *t*-test or the one-way ANOVA followed by the Tukey *post hoc* test was used. The influence of possible confounders, age, sex, AD and *FLG* mutations, respectively, was assessed using multiple linear regression models, where the log-transformed data of DTI, filaggrin and NMF were used as dependent variables. The exponentiated coefficient was reported as % change from the reference variable in the linear regression. Our *a priori* hypothesis was that DTI would be higher, and the level of monomeric filaggrin and NMF lower, in dermatitis patients compared to healthy controls. Furthermore, we expected that patients with *FLG* mutations and a history with AD would have a higher DTI and lower levels of monomeric filaggrin and NMF, compared to patients without these features. *P*-values <0.05 were considered to be statistically significant. Due to the explorative and experimental design of the study, no correction of multiple comparisons was performed. All statistical analyses were performed with IBM SPSS Statistics 22 (IBM, Armonk, NY, USA) and GraphPad Prism 7 (GraphPad Software, Inc, San Diego, CA, USA).

Results

The patient population consisted of 18 males and 29 females, and the median age was 41 years (range 18–67). The control group had 10 male and 10 female participants, and the median age was 29 years (range 20–68 years). No statistical difference in age was found between the patient population and the healthy controls (*P* = 0.553). In the patient population, 78.7% (37/47)

had a history of AD and 59.6% (28/47) had at least one *FLG* mutation (20 heterozygotes and eight homozygotes). Furthermore, 61.7% (29/47) had a history of asthma or hay fever, 29.8% (14/47) had clinical xerosis, 66.0% (31/47) had keratosis pilaris, 63.8% (30/47) had palmar hyperlinearity, and 44.7% (21/47) had a history of fissures on hands and feet. In the control group, 15.0% (3/20) had a history of asthma or hay fever, and 25.0% (5/20) had keratosis pilaris. None of the participants in the control group had clinical xerosis, palmar hyperlinearity or a history of fissures on hands and feet. The median values of DTI, filaggrin and NMF in the patient population and in the healthy controls according to study population characteristics are listed in Table 1.

Dermal Texture Index (DTI)

Overall, the patient population had significantly higher DTI values compared to the control group (median DTI; 25th/75th percentile, 75; 59/98 vs. 44; 31/78, *P* = 0.015; Fig. 2). In the control group, DTI was significantly higher in the oldest age group (>50 years) compared to the youngest age group (<30 years; *P* < 0.0001) and those between 30 and 50 years of age (*P* < 0.0001; Fig. 3). When adjusting for age and sex, the oldest age group had 74% higher DTI values compared to the youngest age group (*P* < 0.0001). In the patient population, no association between DTI and age was found. No significant differences in DTI were found for the other population characteristics in the patient population or the control group in the unadjusted analyses, or after adjustment for age and sex.

Epidermal monomeric filaggrin

Overall, the patient population had significantly lower levels of monomeric filaggrin compared to the control group (AU/mg epidermis; 25th/75th percentile, 58.7;16.5/180.5 vs. 195.2; 108.1/486.3, *P* = 0.001; Fig. 2). In the patient population, participants with *FLG* mutations had significantly lower levels of monomeric filaggrin in the unadjusted analyses (*P* < 0.0001) (Table 1), as previously shown by Bandier *et al.*,⁶ and this was also evident after adjustment for age and sex (*P* < 0.0001). When stratified for the number of mutations, heterozygous mutation carriers had 35% lower levels and the homozygous mutation carriers had 90% lower levels of monomeric filaggrin compared to wild types in the age- and sex-adjusted analyses (*P* = 0.009 and *P* < 0.0001, respectively). Monomeric filaggrin levels were significantly lower in participants with xerosis and palmar hyperlinearity compared to those without such clinical characteristics in the unadjusted analyses (Table 1). Analyses adjusted for age and sex showed that xerosis was associated with a 65% reduction (*P* = 0.001) and palmar hyperlinearity with a 59% reduction (*P* = 0.002) of filaggrin levels. No significant differences in the levels of filaggrin were found with regard to age or sex in the patient population or in the control group.

Table 1 The DTI, level of monomeric filaggrin (AU/mg epidermis) and NMF (mmol/g protein) in dermatitis patients and healthy controls, stratified by study population characteristics

	Patient population† % (n/total)	Controls‡ % (n/total)	DTI¶ median (25th/75th percentile)		Patient vs. Controls P-value††	Filaggrin (AU/mg epidermis) median (25th/75th percentile)		Patient vs. Controls P-value‡‡	NMF (mmol/g protein)§ median (25th/75th percentile)		Patient vs. Controls P-value†††
			Patient population	Controls		Patient population	Controls		Patient population (n = 47)	Controls (n = 20)	
Overall			75 (59/98)	44 (31/78)	0.015	58.7 (16.5/180.5)	195.2 (108.1/486.3)	0.001	0.66 (0.47/0.96)	1.01 (0.78/1.17)	0.005
Sex											
Male	38.3 (18/47)	50.0 (10/20)	85 (60/150)	43 (32/109)	0.090	64.2 (11.3/193.8)	171.8 (93.9/527.3)	0.061	0.64 (0.47/0.89)	0.77 (0.45/1.05)	0.476
Female	61.7 (29/47)	50.0 (10/20)	70 (47/98)	44 (30/59)	0.049	44.1 (19.8/153.0)	207.5 (102.9/388.3)	0.015	0.67 (0.43/0.99)	1.05 (0.89/1.80)	0.001
P-value	—	—	0.197	0.508	—	0.793	0.775	—	0.905	0.029	—
Age group											
<30 years	34.0 (16/47)	55.0 (11/20)	67 (51/82)	33 (29/44)	0.025	49.5 (12.6/227.1)	211.6 (102.7/523.9)	0.026	0.59 (0.46/0.69)	0.99 (0.82/1.11)	0.002
30–50 years	48.9 (23/47)	20.0 (4/20)	86 (55/114)	45 (25/63)	0.023	44.1 (13.7/180.5)	480.7 (40.2/900.0)	0.116	0.72 (0.40/1.01)	1.09 (0.87/1.60)	0.052
>50 years	17.0 (8/47)	25.0 (5/20)	67 (59/87)	114 (93/139)	0.025	100.2 (27.6/172.2)	156.6 (128.1/280.4)	0.118	0.99 (0.83/1.28)	0.77 (0.39/—)	0.564
P-value	—	—	0.553	<0.0001	—	0.812	0.980	—	0.023	0.679	—
History of asthma or hay fever											
No	38.3 (18/47)	85.0 (17/20)	75 (62/92)	47 (33/100)	0.392	51.7 (23.1/127.5)	187.0 (113.6/448.8)	<0.0001	0.81 (0.58/1.10)	1.04 (0.77/1.47)	0.246
Yes	61.7 (29/47)	15.0 (3/20)	73 (48/134)	30 (20/—)	0.012	58.7 (9.0/209.1)	211.6 (36.8/—)	0.232	0.64 (0.39/0.78)	0.99 (0.86/—)	0.062
P-value	—	—	0.191	0.107	—	0.526	0.956	—	0.020	0.924	—
TEWL (g/m²/h)											
Low (<12)	42.6 (20/47)	65.0 (13/20)	79 (41/118)	40 (29/88)	0.055	51.7 (18.1/183.1)	131.6 (81.4/409.6)	0.039	0.72 (0.61/1.02)	1.05 (0.90/1.78)	0.009
High (≥12)	57.4 (27/47)	35.0 (7/20)	69 (59/90)	47 (37/87)	0.219	58.7 (13.7/150.5)	211.6 (187.0/523.9)	0.010	0.63 (0.43/0.92)	0.81 (0.44/1.07)	0.413
P-value	—	—	0.700	0.701	—	0.914	0.347	—	0.327	0.033	—
Keratosis pilaris											
No	34.0 (16/47)	75.0 (15/20)	86 (63/105)	45 (31/109)	0.030	31.8 (10.3/175.6)	211.6 (124.6/537.7)	0.003	0.67 (0.47/0.99)	1.05 (0.81/1.76)	0.025
Yes	66.0 (31/47)	25.0 (5/20)	67 (47/93)	44 (29/47)	0.074	69.7 (23.1/180.5)	125.0 (69.7/363.6)	0.208	0.65 (0.41/0.95)	0.92 (0.61/1.08)	0.285
P-value	—	—	0.310	0.271	—	0.559	0.392	—	0.836	0.312	—
History of AD											
No	21.3 (10/47)	—	67 (60/87)	—	—	100.2 (23.8/172.2)	—	—	0.94 (0.66/1.19)	—	—
Yes	78.7 (37/47)	—	78 (51/108)	—	—	44.1 (9.0/187.8)	—	—	0.63 (0.41/0.80)	—	—
P-value	—	—	0.786	—	—	0.659	—	—	0.027	—	—
FLG mutations											
No	40.4 (19/47)	—	83 (62/108)	—	—	150.5 (41.1/351.7)	—	—	0.73 (0.47/0.95)	—	—
Yes	59.6 (28/47)	—	67 (49/98)	—	—	23.6 (2.5/116.1)	—	—	0.64 (0.46/1.00)	—	—
P-value	—	—	0.556	—	—	<0.0001	—	—	0.872	—	—
FLG mutations											
Wild-type	40.4 (19/47)	—	83 (62/108)	—	—	150.5 (41.1/351.7)	—	—	0.73 (0.47/0.95)	—	—
Heterozygote	42.6 (20/47)	—	71 (57/138)	—	—	64.5 (23.1/134.7)	—	—	0.72 (0.59/1.01)	—	—

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AD, atopic dermatitis according to the UK Working Party's diagnostic criteria; AU, arbitrary units; DTI, Dermal Texture Index; *FLG*, filaggrin gene mutation; NMF, natural moisturizing factors; TEWL, transepidermal water loss.

Low and high TEWL values were defined as values below or above the median value of all participants. Heterozygote: Carriers with one functional filaggrin gene copy. Homozygote: Carriers with double-allele null mutations either compound heterozygous or homozygous. The filaggrin gene mutations analysed are R501X, 2282del4, R2447X. Highlighted in bold are the significant results ($P < 0.05$).

†Independent samples *t*-test (2 groups) or one-way ANOVA (>2 groups) with log-transformed values of NMF and DTI was used to test the statistical difference according to study population characteristics within the patient population. For filaggrin, the Mann-Whitney *U*-test (two groups) or Kruskal-Wallis test (>2 groups) was used.

††Independent samples *t*-test (2 groups) or one-way ANOVA (>2 groups) with log-transformed values of DTI, filaggrin and NMF was used to test the statistical difference according to study population characteristics within the healthy controls.

‡NMF values from 17 participants are missing.

‡†DTI values from five participants are missing.

‡‡Independent samples *t*-test with log-transformed values of NMF and DTI.

‡‡‡Mann-Whitney *U*-test.

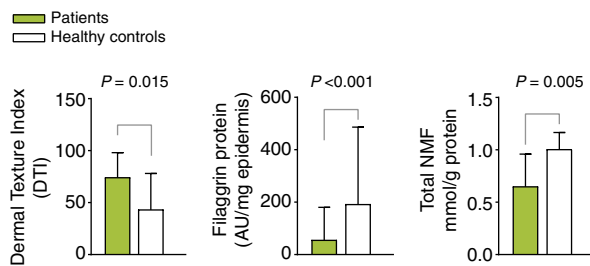


Figure 2 The DTI, levels of monomeric filaggrin (AU/mg epidermis) and NMF (mmol/g protein) in dermatitis patients and healthy controls expressed as median values with interquartile range. For DTI and NMF, independent samples *t*-test with log-transformed values was used to test the statistical difference between the two groups, while for monomeric filaggrin, the Mann–Whitney *U*-test was used. *P*-values <0.05 were considered significant. AU, arbitrary units; DTI, Dermal Texture Index; NMF, natural moisturizing factors.

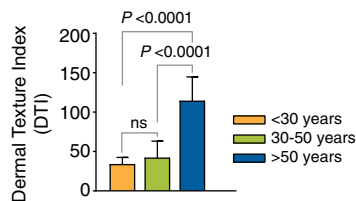


Figure 3 The DTI in healthy participants stratified by age group, expressed as mean (±SD). One-way ANOVA followed by the Tukey *post hoc* test was used to test the statistical difference between the groups. *P*-values <0.05 were considered significant. DTI, Dermal Texture Index.

Filaggrin degradation products (NMF)

Overall, the levels of NMF were significantly lower in the patient population compared to the control group (mmol/g protein; 25th/75th percentile, 0.66; 0.47/0.96 vs. 1.01; 0.78/1.17, $P = 0.005$) (Fig. 1). In the patient population, the NMF levels were significantly lower in participants with a history of AD compared to participants without AD ($P = 0.027$), and when adjusted for age, sex and *FLG* mutations, a history of AD was associated with an 18% reduction in NMF ($P = 0.045$). In the unadjusted analyses, no difference was seen for the presence of any *FLG* mutation, but when stratified for the number of mutations, homozygous carriers had significantly lower NMF levels compared to heterozygous ($P = 0.004$) and wild-type cases ($P = 0.020$). When adjusted for age and sex, homozygous carriers had a 24% reduction in NMF levels compared to wild-type cases ($P = 0.010$). A history of asthma or hay fever was associated with significantly lower levels of NMF in the unadjusted analyses ($P = 0.020$) and after adjustment for age and sex (17%

reduction, $P = 0.015$). Furthermore, xerosis was associated with lower levels of NMF when adjusted for age and sex (16% reduction, $P = 0.025$). With regard to sex, no significant differences in the NMF levels were found. The oldest age group (>50 years) had higher NMF levels compared to the youngest age group (<30 years) in the unadjusted analyses ($P = 0.017$), and when adjusted for sex, a history of AD and *FLG* mutations, the oldest age group had 28% higher NMF levels compared to the youngest age group ($P = 0.023$). In the control group, male participants had significantly lower levels of NMF compared to female participants ($P = 0.029$) (Table 1). Finally, participants with high TEWL values (≥ 12 (g/m²)/h), defined as higher than the median value of all participants, had significantly lower levels of NMF compared to those with lower TEWL values ($P = 0.033$).

Discussion

Main findings

The 47 patients with dermatitis under control had significantly higher levels of DTI and significantly lower levels of monomeric filaggrin and NMF compared to the 20 healthy controls. In these patients, reduced levels of monomeric filaggrin and NMF seemed to correlate with the presence of *FLG* mutations and clinical phenotypes such as xerosis, palmar hyperlinearity and history of AD. Among healthy controls, DTI was significantly higher in the oldest age group compared to the two younger age groups.

Interpretation

The increased DTI observed in patients with dermatitis is in line with previous studies that investigated the relationship between changes in corneocyte morphology and the presence of dermatitis. In 18 healthy newborns followed from birth and up to 6 months, study subjects ($n = 5$) who developed dermatitis had a tendency of higher number of ‘villus-like projections’ compared to those who did not develop AD.² Furthermore, elevated levels of DTI have been found in both lesional (529 ± 277 counts) and non-lesional (116 ± 53 counts) paediatric AD skin when compared to healthy controls (24 ± 21 counts).³ In our study, the observed difference between the patient population and the healthy controls was not as pronounced, which could be explained by the absence of active dermatitis and furthermore that the individuals were adults.

To our knowledge, we are the first to describe significantly increasing levels of DTI with age in healthy skin. A small study including 21 healthy individuals aged 1–77 years found no correlation between DTI and age.³ Nevertheless, morphological changes in aged corneocytes have been described previously. Thus, aged corneocytes compared to young corneocytes have significantly increased cell size, a rougher cell surface and possibly corneocyte protrusions, described by Gorzelanny *et al.*¹⁴ as ‘humps’. The authors could not explain the nature of these

nano-scale alterations, but hypothesized that they were due to decreased production of lipids and epidermal proteins involved in the keratinization process. Previous studies have shown that aged skin has decreased levels of lipids and down-regulation of important epidermal proteins such as filaggrin and loricrin compared to young skin, supporting this assumption.^{15–17} In the current study, we did not observe a significant decrease in levels of filaggrin monomers with age. This suggests that other factors than monomeric filaggrin deficiency are responsible for the high DTI observed in aged healthy skin. For example, we did not measure amount or distribution of lipids around the cells which could account for the difference with age. No association between age and DTI was found in the patient population; however, we observed significantly higher levels of NMF in older patients compared to the two younger age groups ($P = 0.023$), perhaps due to a compensatory breakdown of filaggrin to keep the skin hydrated.

The nature of the altered topography of the corneocyte surface is still unresolved, but several hypotheses have been proposed. While a strong inverse correlation between DTI and NMF was shown in children with acute and convalescent AD,⁴ it cannot be out ruled that skin inflammation associated with AD affected this relationship. However, the DTI level was more closely related to NMF than the SCORAD (SCORing Atopic Dermatitis) value, suggesting that the absence of filaggrin or NMF could be more important for altered corneocyte morphology than inflammation *per se*. This notion was supported by the fact that the DTI was not affected by a change in SCORAD.⁴ Moreover, the level of skin hydration might be important for DTI levels, as suggested in a study by a Matsumoto *et al.*¹⁸ Here, experimentally induced xerosis and inflammation by exposure to the potent contact allergen 2,4,6-trinitrochlorobenzene led to corneocyte protrusions (villi), which disappeared after treatment with moisturizers. In the current study, we were unable to detect a significant difference in DTI levels according to a history of AD, the presence of *FLG* mutations, xerosis or elevated TEWL in the patient population. This finding could be due to the heterogeneity of the patient population, use of emollients or the presence of subclinical inflammation.

The reduced levels of epidermal monomeric filaggrin and NMF in the patient population compared to the healthy controls are in accordance with previous research. Several inflammatory mediators, such as IL-4, IL-13, IL-17A, IL-22, IL-25, IL-33, TSLP and TNF- α , have been shown to down-regulate filaggrin expression,^{5,19,20} even in non-lesional atopic skin.²¹ Our study subjects had no or mild dermatitis which was under control, hereby suggesting that even subclinical inflammation could negatively affect the skin barrier and in turn reduce filaggrin and NMF levels. As expected,^{22,23} the levels of NMF were lower in participants with a history of AD and in carriers of common *FLG* mutations.

Strengths and limitations

To our knowledge, this is the first study to investigate DTI in a relatively large population of adults with and without dermatitis, and also the first study to examine *FLG* mutations, monomeric filaggrin levels and NMF in the same group. There are some limitations with regard to the study design. The biopsy for monomeric filaggrin quantification and the TEWL measurements were taken on the inner surface of the upper arm, while the tape strips used to determine DTI and NMF were collected from the volar aspect of the forearm close to the elbow, possibly leading to bias regarding anatomical variation. Also, the analytical variability of the DTI measure has not yet been established. It is possible that absolute levels of DTI and NMF were reduced as tape strips had been stored since 2011, but we do not expect the relative levels to have been affected. No information on current AD was collected, and no clinical scoring of eczema severity was performed. No correction for multiple comparisons was performed despite a high number of association tests. Significant results should therefore be interpreted with caution as they may be based on chance. Finally, we had no information about emollient use, sun exposure or bathing habits within weeks before sampling.

Conclusion

In this human adult study, a significant difference in DTI, epidermal monomeric filaggrin and NMF was found between patients with a history of dermatitis and healthy controls, indicating that mild dermatitis, or even subclinical inflammation, is sufficient to produce alterations in important skin barrier properties. DTI was significantly increased in aged individuals in the healthy control group, suggesting a steady change in corneocyte morphology with age. Future studies need to identify the nature of DTI-related corneocyte changes including possible elicitors for a better understanding of interplay between cells and skin matrix in the etiopathogenesis of AD and other forms of dermatitis.

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