Stratum corneum lipids, skin barrier function and filaggrin mutations in patients with atopic eczema

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Abstract

Background: Prior to the discovery of filaggrin (FLG) mutations, evidence for an impaired skin barrier in atopic dermatitis (AD) has been documented, and changes in ceramide profile, altered skin pH and increased trans-epidermal water loss (TEWL) in patients with AD have been reported. Until now, no studies have analysed stratum corneum (SC) lipids combined with skin barrier parameters in subjects of known FLG genotype.

Methods: A cohort of 49 German individuals genotyped for the most common FLG mutations (R501X, 2282del4) had SC samples taken for lipid analysis by high-performance thin layer chromatography. In addition, TEWL, erythema, skin hydration and pH were measured. In 27 of the 49 individuals, a 24-h irritation patch test with sodium lauryl sulphate was performed. For the analysis, both the AD group and the control group were stratified by FLG mutation status (FLGmut/FLGwt).

Results: In the FLGmut AD group, significantly lower levels of ceramide 4 and significantly higher levels of ceramide 7 were observed when compared to both healthy control groups. However, ceramide 7 levels also significantly differed between FLGwt AD and FLGwt controls, as did ceramide 1 levels. No significant differences were observed for ceramide 2, 3, 5 and 6. FLGmut individuals had significantly higher skin pH values than individuals not carrying FLG mutations. Patients with AD with FLG mutations had significantly higher erythema compared to patients with AD without FLG mutations.

Conclusion: Our results confirm previous observations of altered ceramide levels in AD, which however appear to show no clear relationship with FLG mutations.

The discovery of loss-of-function mutations within the filaggrin (FLG) genes as major risk factors has highlighted the importance of epidermal barrier dysfunction in atopic dermatitis (AD) (1). A strong and significant association between FLG null alleles and AD, in particular early-onset and persistent AD, has been documented in multiple case-control, family- and cross-sectional studies (2). The presence of FLG mutations (FLGmut) also appears to cause a strong predisposition for asthma in the context of AD (2). Additional associations between FLG mutation and total and specific IgE levels as secondary traits (3) and with rhinitis (4) have been proposed.

The epidermal barrier function critically depends on a proper stratum corneum (SC) formation and composition. Previously, particular attention has been paid to the lipid content of the SC, with data suggesting a prominent role for the SC ceramide profile (5, 6).

Filaggrin mutations preventing the production of free FLG in the epidermis are estimated to occur in up to 10% of the normal population, whereas they are found in up to 50% of patients with moderate to severe AD (2). However, the mechanisms through which FLG mutations contribute to AD risk, and the exact nature of the skin barrier defect associated with FLG deficiency, are not yet understood.
fully understood. Previous studies, conducted prior to the identification of FLG, have provided considerable functional evidence for an impaired skin barrier function in AD, when compared to healthy individuals, as for example increased basal trans-epidermal water loss (TEWL) (7, 8), and differences in SC lipid composition, packaging and processing (9–16). In vitro, it has been demonstrated that the spongilipid metabolism is influenced by changes in keratinocyte differentiation, and that this may lead to changes in the lipid profile (17).

Recently, a correlation between TEWL and FLG-related AD has been noted, with unexpected lower TEWL values for the patients with AD carrying FLG mutations (AD FLGmut) when compared to patients with AD without FLG mutations (AD FLGwt) (18). Another recent study has shown that FLGmut individuals had reduced levels of natural moisturizing factors (NMFs), when compared to noncarriers (19). So far, nothing is known about a possible link between FLG mutations and lipid metabolism. To examine this important aspect of the skin barrier in AD, and to further establish the characteristics of FLG-related AD, we analysed SC lipids and parameters of skin barrier function in subjects with known FLG genotype.

Material and methods

Study cohort

The study was approved by the Ethics Commission of the Bavarian Medical Chamber (Bayerrische Landesärztekammer, No. 08124), and informed consent was obtained from all participants. Four groups of German adult volunteers (32 women, 17 men, median age 49 years) with known FLG status [R501X, 2282del4, typing performed as described previously (4)], were examined: (i) 12 patients with AD carrying FLG mutations (three compound heterozygote, nine heterozygotes, AD FLGmut); (ii) 19 patients with AD not carrying FLG mutations (AD FLGwt); (iii) six healthy individuals with no history of AD or allergic diseases carrying FLG mutations (all heterozygotes, controls FLGmut); (iv) 12 healthy individuals with no history of AD or allergic diseases not carrying FLG mutations (controls FLGwt). Patients with AD enrolled did not suffer from any other major skin disease. All participants were asked not to use any emollients or any topical agents on their forearms 24 h prior to the examination. Only individuals with nonlesional mid-volar forearm were enrolled.

Measurements and scoring

A SC sample for lipid analysis was taken using the cyanoacrylate method (20), where the forearm is wiped with acetone to eliminate contamination from surface lipids, and a drop of cyanoacrylate tissue glue (LiquiBand®, Medlogic Global Ltd, Plymouth, UK) is placed on a glass-slide and held against the skin until the glue dries, and then removed and kept frozen until further analysis with high-performance thin layer chromatography (HPTLC).

Skin lipids were extracted from the cyanoacrylate by sonication in n-hexane: ethanol [95 : 5 (v:v)]. For HPTLC, the skin lipids were separated on silica coated HPTLC plates, using a solvent mixture of chloroform:methanol:acetic acid [190 : 9 : 1 (v:v:v)]. The plates were first dried, and then stained with the florescent probe primuline and the components were quantified through determination of fluorescence intensity, compared with a standard curve made from ceramide 5 and cholesterol. The fluorescence intensity was within the range of standard curve in all quantified samples, and the standard curve was analysed on the same HPTLC plates as the samples (Fig. 1) (20). This setup gives results concerning relative differences in ceramide subgroups and ceramide/cholesterol ratio, but does not give differences in the total amount of lipids. The method mostly quantifies unsaturated fatty acids, which are very limited in SC, and therefore it does not give a valid result for free fatty acids.

We used the simple nomenclature for ceramides (ceramide 1–9); but to avoid any misunderstandings, we have added the nomenclature proposed by Motta in Fig. 2, where the structure of the ceramides are described as follows: A, α-hydroxy fatty acid; EO, ester-linked ω-hydroxyl acid; N, nonhydroxy fatty acid; P, phytosphingosine; S, sphingosine; H, 6-hydrosp-hingosine (21).

Skin pH measurements were carried out on nonlesional skin on the extensor forearm, using a skin pH meter (PH 900; Courage and Khazaka, Cologne, Germany). Three recordings from each test area were performed, and the mean value was calculated and used for further analysis. Severity of AD was evaluated using the SCORAD (SCORing Atopic Dermatitis) (22).

All following measurements were performed on nonlesional skin at the mid-volar forearm.

Basal levels of TEWL, erythema and electrical capacitance were measured on day 1. In 27 individuals, a 24-h irritation test with sodium lauryl sulphate (SLS) was performed using large Finn chambers® (diameter 12 mm) with 60 μl SLS (> 99% purity; Sigma Chemical Co., St. Louis, MO, USA) (23), in a 1% aqueous solution on filter discs. Trans-epidermal water loss and erythema were measured again on day 3, which gave delta TEWL (TEWL after the 24 h SLS irritation, minus TEWL before the SLS irritation) and delta erythema (erythema after the 24 h SLS irritation, minus erythema before the SLS irritation).

The TEWL (g/m² per hour) measurements were performed in accordance with established guidelines (24). Erythema measurements were performed with a Minolta Chroma Meter CR-300 (Minolta Camera, Osaka, Japan). The colour is expressed in the L*a*b system, where erythema of the skin is indicated by a* (25).

For electrical capacitance, a Corneometer (CM 825; Courage and Khazaka, Köln, Germany) was used. The unit for capacitance is arbitrary, but correlates to the water content of the skin (26).

For all above-mentioned noninvasive measurements, two measurements were made, and the mean value was calculated. If the two TEWL values measured were more than
1.5 g/m² per hour apart, the participant was asked to relax another 5 min, before the measurement was repeated.

Statistical analysis
For comparing the distribution of quantitative parameters we used the nonparametric Kruskall–Wallis test as omnibus test for more than two groups. In case of significance we performed pairwise comparison of the groups with the Wilcoxon two-sample test.

P-values <0.05 were considered statistically significant. We did not correct for multiple testing and our analysis has to be considered explorative.

Results
Significant differences of ceramide 1, 4 and 7 between AD and controls
The percentage distributions of the different ceramides are seen in Fig. 2 and Table 1. Significant differences between the four groups were observed for ceramide 1, 4 and 7 (P = 0.043, 0.030, 0.008) using the Kruskall–Wallis test. For ceramide 1, the FLGwt AD group had significantly lower levels when compared to the FLGmut healthy group. For ceramide 4, the FLGmut AD group had significantly lower levels compared to both healthy groups. For ceramide 7, we found significantly higher levels in the FLGmut AD group when compared to both healthy control groups. For ceramide 7, a significantly higher level was also observed in the FLGwt AD group when compared to the FLGwt healthy control group (Table 1). We accumulated the two groups with FLGmut and the two groups with FLGwt for ceramide 4 and ceramide 7 and found no statistically significant differences (P = 0.20, 0.72).

No significant differences between the 4 groups were observed regarding ceramide 2, 3, 5 and 6 levels.

With respect to the ceramide/cholesterol ratio, no significant difference between any of the groups was found.

Differences in TEWL, erythema, electrical capacitance and skin pH for FLG-related AD versus nonFLG-related AD and controls
Results from the measurement of skin barrier function parameters are presented in Table 2.

For baseline TEWL, significant differences between the groups were found (P = 0.042), with higher values for AD (FLGmut) than for healthy controls. We observed higher baseline TEWL values in the FLGmut groups (6.1 vs 5.7 for the AD groups and 5.8 vs 4.6 for the healthy groups), although not statistically significant (P > 0.05 (Table 2).

The delta TEWL values, before and after 24 h SLS irritation, again presented higher values in the FLGmut groups, although not statistically significant.

For basal erythema, significant differences between groups were found (P = 0.001), with the most erythema for the FLGmut AD compared to groups with FLGwt.

The delta values for erythema intensity from before and after 24 h SLS irritation are presented in Table 2. No statistically significant differences were found between groups.

The skin hydration, evaluated by capacitance measurements are presented in Table 2, and significant differences were found between groups (P = 0.006). The differences were significant for the FLGmut AD group versus FLGwt controls (P = 0.0047) and for FLGmut controls versus FLGwt controls (0.035).

pH measurements demonstrated significant differences between groups (P = 2.6e-6). In general, FLGmut individuals had higher values than FLGwt individuals, and the highest values were observed in the FLGmut atopic group (Table 2) (Fig. 3).
SCORAD in FLG-related AD and nonFLG-related AD

The clinical evaluation SCORAD of the people with eczema is presented in Table 2. No significant difference between FLG mut AD and FLG wt AD for clinical severity of AD was found, although we did observe slightly higher scores in the FLG mut group.

Discussion

Atopic dermatitis is a common chronic inflammatory skin disease, which is strongly influenced by genetic factors (27). Some of its hallmark features are dry, itchy skin, a marked permeability barrier abnormality and SC abnormalities (28, 29). Null mutations in the FLG gene leading to a diminished production of free FLG in the epidermis are the most widely replicated and strongest known risk factors for AD. Up to 50% of patients with AD suffer from an inherited FLG deficiency. However, the functional consequences and exact phenotypic characteristics caused by FLG mutations are not yet completely understood. In particular, the relationship between FLG status and previously identified lipid abnormalities relating to SC function is of interest. Several studies examined differences in SC lipids and barrier function between the patients with AD and healthy controls (9, 10, 13, 14), but so far the relationship between FLG status and SC

| Table 1 Results of the percentage distribution of ceramide 1–7 and ceramide/cholesterol ratio in atopic dermatitis and healthy individuals with known filaggrin (FLG) status |
|---------------------------------|---------------------------------|---------------------------------|---------------------------------|
| Ceramides | Measurement (median values with 1. and 3. quartile in brackets) | People in each group, N | Significance using the Kruskal-Wallis test | Significance using Wilcoxon two sample test |
| Ceramide 1 | Gr.1: 13.4 (12.2–15.1) 12 | P = 0.043 | Significance was found between. group 2 and 4 (P = 0.012) |
| | Gr.2: 12.6 (11.1–15.0) 19 | | |
| | Gr.3: 14.9 (13.6–16.0) 6 | | |
| | Gr.4: 16.9 (14.4–17.7) 12 | | |
| Ceramide 2 + 9 | Gr.1: 20.3 (18.0–22.4) 12 | P > 0.05 | Not performed |
| | Gr.2: 20.0 (16.2–20.8) 19 | | |
| | Gr.3: 18.9 (18.6–19.7) 6 | | |
| | Gr.4: 18.4 (17.7–20.0) 12 | | |
| Ceramide 3 | Gr.1: 17.2 (16.1–18.2) 12 | P > 0.05 | Not performed |
| | Gr.2: 17.9 (14.7–21.3) 19 | | |
| | Gr.3: 20.4 (19.0–22.2) 6 | | |
| | Gr.4: 18.4 (16.2–20.8) 12 | | |
| Ceramide 4 | Gr.1: 6.0 (4.1–7.3) 12 | P = 0.030 | Significance was found between group 1 and 4 (P = 0.006) and group 1 and 3 (P = 0.032) |
| | Gr.2: 6.4 (4.8–8.7) 19 | | |
| | Gr.3: 8.7 (7.0–9.2) 6 | | |
| | Gr.4: 7.8 (6.9–9.6) 12 | | |
| Ceramide 5 + 8 | Gr.1: 21.9 (19.1–22.9) 12 | P > 0.05 | Not performed |
| | Gr.2: 20.4 (18.1–21.3) 19 | | |
| | Gr.3: 18.7 (18.5–19.0) 6 | | |
| | Gr.4: 18.2 (16.7–19.6) 12 | | |
| Ceramide 6 | Gr.1: 9.1 (8.2–10.8) 12 | P > 0.05 | Not performed |
| | Gr.2: 10.5 (8.6–13.3) 19 | | |
| | Gr.3: 8.6 (8.3–9.3) 6 | | |
| | Gr.4: 9.5 (7.2–11.5) 12 | | |
| Ceramide 7 | Gr.1: 12.5 (11.7–13.8) 12 | P = 0.008 | Significance was found between group 1 and 4 (P = 0.009) and group 1 and 3 (P = 0.016) and group 2 and 4 (P = 0.01) |
| | Gr.2: 12.1 (10.1–14.6) 19 | | |
| | Gr.3: 10.1 (9.8–10.4) 6 | | |
| | Gr.4: 9.9 (9.4–11.4) 12 | | |
| Ceramide/cholesterol ratio | Gr.1: 1.55 (1.28–1.83) 12 | P > 0.05 | Not performed |
| | Gr.2: 1.60 (1.45–1.90) 19 | | |
| | Gr.3: 1.60 (1.50–2.08) 6 | | |
| | Gr.4: 1.55 (1.30–1.78) 12 | | |

Group 1: Eczema and FLG mutations (FLGmut); Group 2: Eczema, no FLG mutations (FLGwt); Group 3: Control and FLG mutations (control mut); Group 4: Control, no FLG mutations (control wt).
lipids has not been investigated, and only two studies determined skin barrier parameters in dependency on FLG status (18, 19).

In this study we examined SC lipids and parameters of skin barrier function in patients with AD, with and without (mut/wt) FLG mutations, and compared them to subgroups of healthy controls, also subdivided according to FLG status. It should be noted that the AD groups versus the control groups do not represent the background population, because they were selected to get additional persons with FLG mutations, therefore comparing patients with AD (both FLGwt and FLGmut) and controls (FLGwt and FLGmut) does not give representative results. In addition, individuals were only genotyped for the two most common FLG mutations R501X and 2282del4. However, as a previous population-based study on more than 3000 German schoolchildren showed that the 2-s next common FLG mutations (R2247X, S3247X) are present in <1% of German individuals (4), we do not think that typing of these variants would have altered our findings significantly.

Levels of ceramide 1 only differed significantly when comparing patients with FLGwt AD to FLGwt controls. In the FLGmut AD group, significantly lower levels of ceramide 4 and significantly higher levels of ceramide 7 were observed, when compared to both healthy control groups. For ceramide 7, we also observed a significant difference between the FLGwt AD and the corresponding healthy group. When combining the two groups with FLGmut (both healthy and AD) and comparing them to the FLGwt individuals, no significant difference was found. These findings imply that levels of ceramide 1, 4 and 7 might be altered in AD in general rather than representing an FLG-related feature. It is of note that this is the first time that ceramide 4 turned out to be significantly lower in patients with AD when compared to healthy individuals, yet only in patients with AD carrying FLG mutations. However, the number of individuals without a history of AD or allergic

**Table 2** Results of skin barrier function parameters in atopic dermatitis and healthy individuals with known filaggrin (FLG) status

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Measurement (median values with 1. and 3. quartile in brackets)</th>
<th>People in each group</th>
<th>Significance using the Kruskal-Wallis test</th>
<th>Significance using Wilcoxon two sample test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Basal TEWL (gm⁻²/h) (N = 49)</td>
<td>Gr.1: 6.1 (5.4–8.1) Gr.2: 5.7 (4.9–10.5) Gr.3: 5.8 (5.0–6.8) Gr.4: 4.6 (3.8–5.4)</td>
<td>12</td>
<td>P = 0.042</td>
<td>Significance was found between group 1 and 4 (P = 0.0042)</td>
</tr>
<tr>
<td>Delta TEWL (gm⁻²/h) (N = 27)</td>
<td>Gr.1: 17.6 (12.6–22.9) Gr.2: 8.9 (7.7–17.3) Gr.3: 25.5 (22.7–25.7) Gr.4: 17.1 (10.1–24.5)</td>
<td>6</td>
<td>P &gt; 0.05</td>
<td>Not performed</td>
</tr>
<tr>
<td>Basal erythema (N = 49)</td>
<td>Gr.1: 10.9 (9.1–13.8) Gr.2: 9.3 (8.0–10.1) Gr.3: 7.6 (7.5–7.9) Gr.4: 8.2 (7.4–8.8)</td>
<td>12</td>
<td>P = 0.001</td>
<td>Significance was found between group 1 and 2 (P = 0.027) and group 1 and 4 (P = 0.0042)</td>
</tr>
<tr>
<td>Delta erythema (N = 27)</td>
<td>Gr.1: 4.0 (3.2–5.5) Gr.2: 4.0 (3.9–5.2) Gr.3: 6.9 (4.4–7.0) Gr.4: 5.6 (4.1–8.8)</td>
<td>6</td>
<td>P &gt; 0.05</td>
<td>Not performed</td>
</tr>
<tr>
<td>Capacitance (N = 27)</td>
<td>Gr.1: 31.5 (26.3–34.3) Gr.2: 35.0 (25.0–41.5) Gr.3: 44.5 (40.0–48.3) Gr.4: 37.0 (35.5–41.7)</td>
<td>12</td>
<td>P = 0.006</td>
<td>Significance was found between group 1 and 4 (P = 0.0047)</td>
</tr>
<tr>
<td>Skin pH (N = 27)</td>
<td>Gr.1: 5.81 (5.77–5.89) Gr.2: 5.60 (5.51–5.67) Gr.3: 5.73 (5.71–5.75) Gr.4: 5.49 (5.47–5.50)</td>
<td>12</td>
<td>P = 2.6e-6</td>
<td>Significance was found between group 1 and 2 (P = 7.9e-5), group 1 and 3 (P = 0.0292), group 1 and 4 (P = 0.0003), group 3 and 4 (P = 0.0014), group 2 and 3 (P = 0.0009)</td>
</tr>
<tr>
<td>SCORAD (N = 31)</td>
<td>Gr.1: 32.9 (19.5–43.1) Gr.2: 28.1 (19.7–42.1)</td>
<td>12</td>
<td>P &gt; 0.05</td>
<td>Not performed</td>
</tr>
</tbody>
</table>

TEWL, trans-epidermal water loss; SCORAD, SCORing Atopic Dermatitis.

Group 1: Eczema and FLG mutations (FLGmut); Group 2: Eczema, no FLG mutations (FLGwt); Group 3: Control and FLG mutations (control mut); Group 4: Control, no FLG mutations (control wt).
Filaggrin mutations, skin barrier and lipids

In addition, increased relative amounts of ceramide 7 related to AD have not been reported before. This is most likely because of the technical improvements, as ceramide 7 could not be differentiated in most earlier studies. The most recent study that compared ceramides in atopic versus healthy skin did differentiate ceramide 7, and although no statistically significant differences were detected, a tendency for lower ceramide 7 in healthy controls when compared to atopics was noted. The study was, however, based on only seven individuals in each group, and a significance level of \( P < 0.01 \) was used (30). Additional larger studies therefore appear warranted.

The ceramide/cholesterol ratio has previously been shown to be lower in patients with AD (13). Our results, with almost no difference between the groups, seem to be in contrast to this, but a recent study has shown significant differences of ceramide/cholesterol ratio between genders, with the highest values for women (Jungersted JM, Hellgren LI, Drachmann T, Hogh JK, Jemec GBE, Agner T, unpublished data). The higher proportion of men in both control groups when compared to both AD groups in our study may therefore have influenced the ceramide/cholesterol findings. In the described study, no gender-related difference was found with respect to percentage distribution of ceramides (Jungersted JM, Hellgren LI, Drachmann T, Hogh JK, Jemec GBE, Agner T, unpublished data).

Basal TEWL measurements revealed a significant difference between individuals suffering from AD and healthy individuals, as have been shown before (7, 8). This illustrates that even nonlesional skin has impaired barrier function in AD. The highest TEWL values were observed in \( FLG \)mut AD, which is in contrast to a recent study of Japanese individuals that found an increased TEWL in patients with \( FLG \)wt AD when compared to \( FLG \)mut AD individuals (18), but in agreement with a larger study on Europeans (19). Given the proposed functions of \( FLG \) (31), the xerotic phenotype of ichthyosis vulgaris and many AD cases (32), and the increased TEWL observed in other types of ichthyosis (33), we think that an increased rate of TEWL in \( FLG \)mut individuals is plausible, and that alternative findings may be explained by individual variation and sample size of the studies.

It is well known that irritants are strong trigger factors of AD (28). In our study, the 24-h SLS irritation test showed a tendency to higher delta TEWL values for the FLG deficient groups (AD and healthy controls) even though not statistically significant. The lack of limited magnitude of the reaction may be because of the relatively low number in each group participating in the irritation test.

Concerning basal erythema, a significant difference was observed between the \( FLG \)mut AD and \( FLG \)wt AD group. As the two AD groups had comparable SCORAD values, this is an interesting finding, because it indicates that basal erythema may be directly linked to \( FLG \) status rather than to severity of the disease. The recent finding that the SC is thicker in people with \( FLG \)mut AD would, however, lead to expectations of less erythema for obvious optical reasons (18). One might speculate whether the higher erythema observed could be attributed to \( FLG \)-related changes in optical qualities of the individual keratinocytes or to an altered SC structure. However, more research is warranted to confirm and clarify this observation.

Skin pH is known to be significantly elevated in patients with AD, even in unaffected areas, and higher in patients with active lesions than in asymptomatic patients (34). Interestingly, in the current study we observed the highest skin pH values in \( FLG \)mut individuals. Skin pH values were notably higher in healthy \( FLG \)mut individuals than in patients with \( FLG \)wt AD, indicating that the elevation of skin pH is related to \( FLG \) status rather than to AD. Filaggrin breakdown products likely contribute to the maintenance of the skin pH (31), and thus lower the levels of acidic breakdown products in \( FLG \)mut individuals could explain the observed increase in skin pH in these individuals.

\( FLG \)mut individuals are associated with lower levels of NMF (19), which contributes to the maintenance of the SC pH, which is in good agreement with the observed increase in pH in \( FLG \)mut individuals in this study. However, it should be noted that the pH measurements were performed using a simple electrode on skin surface, as no new generation device was available at the time of the study. It has been shown that a pH gradient exists through the SC, and thus caution has to be taken in interpreting the data (35).

Different enzymes have a different pH optimum. Ceramide 1 and 4, which we found to be reduced in \( FLG \)mut individuals, belong to a distinct group of ceramides named acylceramides, which are generated trough the enzyme glucosylceramide deacylase (36). However, as the pH optimum for glucosylceramidase...
mide deacylase is near 5, as is the case for most of the other ceramide enhancing enzymes (37), it does not explain the increased level of ceramide 1 and 4 in this study. But considering that our study finds that ceramide 1 and 4 are altered in AD in general rather than being an FLG-related feature, the increased pH of FLGmut individuals has other implications. Enzymes in the SC that have close to neutral pH optimum are the desquamation enhancing serine proteases (34).

For the skin hydration measurements, a tendency towards lower values in patients with AD, particularly those with FLGmut, was found. However, no significant differences were seen between FLGmut AD and FLGwt AD.

The SCORAD values for FLGmut and FLGwt AD did not show any statistically significant differences in this study, which is in line with two other recent studies (18, 38).

In summary, we obtained preliminary evidence that FLG mutations are associated with an increased skin pH. Ceramide levels are altered in patients with AD, but most probably because of the mechanisms other than FLG. In patients with AD with FLG mutations, despite a thicker SC, an increased skin redness was observed when compared to patients with FLGwt AD. Although carefully phenotyped, our study cohort is small and selected, and, future studies of large longitudinal or cross-sectional population-based cohorts that have been typed for all FLG variants and examined for parameters of skin barrier function are needed to reliably contrast FLG phenotypes.

Conflicts of interest
No conflicts of interests.

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