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Title: Skin reaction and regeneration after single SLS exposure stratified by filaggrin genotype and atopic dermatitis phenotype

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What's already known about this topic?

Subjects with AD have barrier defects and increased susceptibility to irritants.

Studies have suggested that filaggrin mutations facilitate penetration of irritants, which could explain an increased risk of ICD among patients with AD and filaggrin mutations.

What does this study add?

Skin response and regeneration after SLS barrier disruption is more depended on AD than on filaggrin genotype.

Those with both mutations and AD have a higher inflammatory alertness, with a more rapid inflammatory response.

Abstract

Background: Filaggrin is key for the integrity of the stratum corneum. Mutations in the filaggrin gene (FLGnull) play a prominent role in the atopic dermatitis (AD) pathogenesis. Persons with AD have an increased susceptibility to irritants. However, little is known about the effect of filaggrin genotype and AD phenotype concerning irritant response and skin regeneration.

Objectives: To investigate the role of FLGnull and AD concerning skin reaction and recovery after Sodium Lauryl Sulfate (SLS) irritation.

Methods: Case-control study comprising 67 subjects, including healthy controls and patients with and without FLGnull and AD. Reactivity to different doses of SLS at 24, 48, 72 and 145 hours after SLS application was measured by transepidermal water loss (TEWL) and laser Doppler flowmetry (LDF). Reactivity was assessed univariately and by pattern analysis technique.

Results: All patient groups showed a higher degree of skin barrier disruption and inflammation than did controls in response to SLS. Assessing reactivity by Δ AUC both TEWL and LDF showed significant difference between healthy controls and those with the AD phenotype irrespective of filaggrin mutation. The poorest regeneration was among those with the AD phenotype.

The two AD phenotype groups were separated by multivariate technique, due to earlier inflammatory reactivity among subjects with FLG^{null}_{plus}AD compared to the AD phenotype alone.

Conclusions: Both skin reaction and regeneration was significantly different between the patient population and the healthy controls. Additionally, response severity and regeneration depended more on phenotype (atopic dermatitis) than on filaggrin genotype, whereas the response was more rapid among FLG^{null}_{plus}AD individuals.

Introduction

Filaggrin is a key epidermal protein primarily located in the stratum corneum. Filaggrin has several complex interrelated functions involving properties of mechanical strength, hydration and resistance, and in regulation of the epidermal homeostasis¹⁻³. Frameshift or nonsense mutations in the epidermal differentiation complex on chromosome 1q21, result in either a partial or total lack of filaggrin dependent on heterozygote or homozygote status⁴⁻⁶, which endogenously influences the skin barrier integrity.

Exogenous substances, such as irritants, commonly affect skin barrier integrity. The severity of the clinical manifestation of dermatitis is influenced by: the concentration and potency of the irritant, the skin contact time, anatomical site, seasonal changes and individual susceptibility⁷⁻¹¹.

In 1982 Malten suggested that the greater the response to irritancy, the poorer the skin regeneration¹². Studies showing increased penetration of water-soluble substances in mice and penetration of dye in an in vitro skin model, suggests that filaggrin deficiency facilitates penetration through the skin barrier^{13;14}. Accordingly, subjects with filaggrin-deficient skin barrier may have a more severe primary response and/or a longer skin recovery after induced irritant reactions; concomitantly, both the skin response and recovery to irritancy would also depend on the AD phenotype, as other studies have shown increased response

to Sodium Lauryl Sulfate (SLS) due to phenotype alone^{15;16}. Only few studies have investigated the skin response and regeneration according to AD phenotype and filaggrin genotype together¹⁷⁻²⁰. Moreover, skin regeneration after SLS barrier disruption has only been investigated in a study with the last measurement on Day 3¹⁸ and in study on mRNA expression of profilaggrin on Day 7²¹.

Hence, we investigated the response to SLS with non-invasive methods in 4 groups: a healthy control group—without AD and without filaggrin mutations; a group without AD and with filaggrin mutations; a group with AD and without filaggrin mutations; and a group with both AD and with filaggrin mutations. We investigated the reaction severity and the following regeneration after barrier disruption and hypothesized that filaggrin mutations would influence the skin barrier function with increased reactivity and decreased skin recovery.

Material Methods

Participants

This case-control observational study comprised 67 individuals, of whom 47 were patients recruited through review of medical files at the department of Dermato-Allergology at Gentofte Hospital Denmark. We recruited 20 healthy controls through online advertising. The distribution of age and sex was equal between all groups.

The pre-sample size to identify significant higher TEWL among FLG^{null}_{plus}AD compared to controls were calculated by online calculator (<http://www.openepi.com>) with 80% power and p-value= 0.05

Table 1 shows the eligibility criteria.

AD was assessed by using the UK Working Party's Diagnostic Criteria²² and filaggrin mutation status for R501X, 2282del4 and 2447X were determined by multiplex analysis²³. Filaggrin homozygosity, compound heterozygosity, and heterozygosity were pooled and analysed as one group (FLG^{null}). None of the patient had Ichthyosis Vulgaris.

All participants received both oral and written information and consent was obtained from all participants. The study was approved by the Ethics Committee of Copenhagen County (project identification: H-1-2011-011) and by the Danish Data Protection Agency.

Distribution of the 4 different groups according to the phenotype AD/non AD and genotype (FLG^{null}/FLG^{wt}) constituted 20 FLG^{wt}_{nonAD} (healthy controls), 10 FLG^{null}_{nonAD}, 19 FLG^{wt}_{plusAD}, and 18 FLG^{null}_{plusAD} as illustrated in Fig. 1.

During the study, 1 participant dropped out on Day 2 and one on Day 8. Their data were addressed as missing those days and forward in the trial.

Test parameters and equipment

To investigate response and regeneration of the skin, measurements reflecting skin barrier were performed; respectively transepidermal water loss (TEWL), dermal inflammation by laser Doppler flowmetry (LDF), and skin pH. Clinical assessment of the irritant reactions due to SLS was performed using the simple scoring system by the ESCD guidelines⁹. The TEWL (g/m²/hour) measurements were performed according to established guidelines²⁴. All probes were calibrated according to fabrication guidelines. Information on the non-invasive bioengineering electronic devices is shown in Table 2 and the experimental protocol presented in Table 3.

Data analysis and statistics

The normal distribution of the data was tested ~~visually and~~ by the Shapiro-Wilks test, showing in general skewed data ($p < 0.05$), thus non-parametrical tests were used (Kruskal-Wallis and Mann-Whitney test). Wilcoxon test was performed to analyse repeated measures of 2 conditions. Chi² statistics was performed to analyse the distribution of the clinical scores by linear-by-linear associations between individual groups.

AUC, Δ reactions and regeneration (R) of the skin was evaluated as described under forms in table 3.

Supplementary to the univariate evaluations of LDF, TEWL, pH, clinical score and the derived delta measures and R, we employed a pattern analysis technique Anova Simultaneous Component Analysis (ASCA)²⁵ for extraction of patterns while sustaining the power of the study design. This was conducted on a matrix with 201 samples (67 by three SLS concentrations) and 26 variables. Multivariate significance testing was by permutation testing with 10.000 permutations examining the effect of SLS concentration and FLG/AD classes. Data were processed in the Statistical Products and Service Solutions package (SPSS

statistics Inc., Chicago, IL, USA; IBM PASW statistics) for Windows, version 19.0 for the univariate analysis, and by Matlab R2014a for MAC (Mathworks® Inc, Natick, MA, USA) for the multivariate analysis.

Results

Of the participants, 28 were men and 39 women, median age 38 years (range 18–68 years). During measurements, the room temperature was kept constant at a mean 24.3 ± 0.83 (range 22.0–26.8) °C and the mean humidity of $36\% \pm 6.23$ (Range 22%-64%).

Assessment of baseline values

Baseline values of TEWL, LD, pH and skin temperature according to phenotype and genotype are illustrated in Fig. 2.

There was no significant difference in overall analysis between groups in either LDF, TEWL, pH or skin temperature between the groups (respectively $p=0.17$, $p=0.066$, $p=0.75$ and $p=0.130$). The results on pH are published elsewhere²⁶. No further notion will be taken on skin temperature.

Skin responses to SLS

Assessment of pH

Variation in pH over time is illustrated in Fig. 3.

Laser Doppler flowmetry and TEWL

Skin reactions from baseline values to the different doses of SLS are illustrated in Fig. 4.

Significant differences in the Δ reactions are shown in Table 4.

At the highest concentration, 1.00% SLS, a significant difference in Δ LDF and Δ TEWL was seen in all 4 groups from baseline to Day 2 and Day 3, assessed by the Wilcoxon signed rank test ($p < 0.005$).

For both TEWL and LDF, maximum responses occurred on Day 3, except for the FLG^{null}_{plus}AD group LDF, where a maximum was reached on Day 2 (Fig. 4). All patient groups displayed a higher LDF and TEWL response compared with healthy controls at the highest SLS concentration (Fig. 4).

When analysing the reaction to different doses of SLS, there was a conspicuous difference on Day 2 primarily between those with the AD phenotype, FLG^{wt}_{plus}AD and FLG^{null}_{plus}AD compared with the healthy controls Table 4.

To evaluate the total response of each individual throughout each dose of SLS, area under the curve (AUC) was calculated and the results are shown in Table 5.

No significant differences between groups on Day 3 were seen in either AUC-LDF or AUC-TEWL.

Clinical scores

All 4 groups displayed a maximum reaction to 1.00% SLS. No significant difference was detected between the groups at the lowest dose of SLS. At the concentration of 0.50% SLS, a significant difference between healthy controls and FLG^{null}_{plus}AD, $P = 0.0056$ was detected on Day 2. At the maximum SLS dose, a significant difference was found between healthy controls and FLG^{wt}_{plus}AD and controls and FLG^{null}_{plus}AD ($p = 0.040$ and $p = 0.00029$ respectively). No difference at either concentrations occurred on Day 3.

Skin regeneration

Laser Doppler Flowmetry and TEWL

For LDF on Day 8, no significant differences between groups were seen at any of the three concentrations. For TEWL, a significant difference between healthy controls and AD individuals irrespective of filaggrin mutation status was seen at the 1.00% SLS site ($p = 0.015$

and $p = 0.024$).

For regeneration R, full regeneration for the healthy controls was seen after exposure to the lowest dose of SLS (0.25%) and poorer regeneration when exposed to 1.00% SLS, displaying a dose-response regeneration. The same pattern was seen in TEWL; however, none of the 4 groups was fully regenerated even at 0.25% SLS (Table 6).

No significant differences in R between groups were detected in any of the 3 concentrations for either LDF or TEWL. For AUC, no significant difference between groups was seen when analysing on Δ AUC LDF, whereas Δ AUC-TEWL significantly differed between healthy controls and FLGwt_{plusAD}, $p = 0.007$.

Clinical Score

A significant difference occurred at both 0.50 and 1.00% SLS between the groups healthy controls and FLGnull_{plusAD} and between FLGwt_{plusAD} and FLGnull_{plusAD}, $p < 0.005$.

Multivariate analysis, pattern analysis

Figure 5a illustrates the loading plot with two components from the ASCA model reflecting the dominating variation in relation to the study design. Component 1, reflecting overall reactivity as all variables loads in the same direction, except for regeneration, which, by construction, inversely relates to its input responses (Figure 5a). Component 2 is primarily driven by LDF Day 1 and Clinical Score Day 2 on the positive side and an increase in TEWL from baseline to Day 2, 3 and 8 on the negative side. Figure 5b illustrates the corresponding scores plot for increasing SLS concentrations. Increasing SLS concentrations resulted in higher responses on component 1 and thus increase in severity ($p_{\text{SLS}} < 10^{-4}$). In line with the univariate measures, the control group obtained the lowest values across all SLS concentrations, whereas the three case groups obtained similar levels on component 1. In component 2 there is significant separation between the FLGnull_{plusAD} and the remaining groups ($p = 0.005$), which primarily was driven by a higher baseline LDF, especially among homozygote/compound heterozygote in the FLGnull_{plusAD} group (N=8) (results not shown). When eliminating the LDF Day 1 from the multivariate model, the inference lessened between the FLGnull_{plusAD} and the remaining groups, though still significant ($p = 0.02$). In

the multivariate model we found that Δ TEWL is the driving discriminator (most pronounced at Day 2 and 3 and for the lower SLS concentrations – see univariates).

Discussion

This study shows that having filaggrin mutations, atopic dermatitis or both results in a higher reaction and poorer regeneration of the skin after SLS provocation compared to normal skin. Filaggrin mutations and AD are closely related and it is known that individuals with AD have a sensitive skin barrier²⁷⁻³⁰.

Baseline results

Surprisingly, we did not find any significant difference in baseline TEWL, LDF or pH between our population groups measured on non-lesional skin sites. This is in keeping with other recent studies showing no significant difference in TEWL at baseline between a control population and subjects with AD, irrespective of filaggrin status^{20;31}, and between patients with AD with or without filaggrin mutations on both lesional and non-lesional atopic skin sites³². Before stratifying for FLGnull, studies have shown increase in TEWL among AD in comparison to controls³³. When stratifying for FLGnull a further significant increase in TEWL has been shown³⁴. Interestingly, a study on Irish children with moderate to severe AD showed an increase in TEWL according to genotype, with the highest TEWL among FLG homozygotes, suggesting increasing TEWL according to filaggrin mutations³⁵.

Other studies have shown significantly higher TEWL values among FLGnull_{nonAD} and FLGnull_{plusAD} compared with healthy controls^{17;18;34}. Thus, an inconsistent result on baseline TEWL exists in the literature.

A reason for these inconsistencies could be due to three things: difference in population sizes, anatomical differences in measurement locations and the fact that AD is a very heterogeneous disease not only based on differentiation by filaggrin mutations.

SLS reaction

SLS is a detergent, and thereby an irritant affecting the skin barrier. SLS is widely used in dermatological research in experimentally induced irritant contact dermatitis^{9;37}. In our study all subjects displayed dose-dependent barrier impairment and inflammation after SLS provocation. This is in line with a study showing similar dose-dependent skin impairment

after application of increasing doses of SLS in healthy subjects³⁷ and more recently, with the findings of Petersen et al showing SLS dose-dependent local inflammation measured by LDF^{40;41}.

In our patient population we measured a more severe skin reaction on Day 2 and Day 3 compared to healthy controls. When viewing the SLS reactions separately, the vascular response on Day 3 suggests that having either phenotype or genotype induces an equal vascular response to irritancy (Fig. 4). For the total reactivity Δ AUC, a significantly higher response was seen on Day 2 among patients with AD irrespective of filaggrin mutations in both Δ AUC LDF and TEWL in comparison to healthy controls. This was in accordance with our clinical assessments, showing that those with the AD phenotype displayed a more severe clinical response to 1.00% SLS than did the healthy controls. This again suggests a reduced threshold to irritants due to AD. Because no significant difference was detected between healthy controls and FLG^{null}_{nonAD} and between FLG^{null}_{plusAD} and FLG^{wt}_{plusAD}, filaggrin mutations alone do not seem to contribute significantly to the irritancy level in this study, as also reported in other studies^{17;20}. An explanation of this could be functional filaggrin defects^{32;42}.

FLG^{null}_{nonAD} had increased response in comparison to controls, though non-significant, which could be due to the small group number (N=10).

Both in mouse studies and larger population studies, the notion that filaggrin deficiency leads to a more susceptible barrier function has been proposed^{7;14;43-45}. However, we were not able to show any increased reactivity among individuals with filaggrin mutations irrespective of AD status by univariate analysis. Other studies also fail to show any increased reactivity according to filaggrin genotype^{17;18}. A possible explanation could be that these studies are based on small study groups and that homozygote and heterozygote individuals are often assessed together. One study in ichthyosis vulgaris patients showed significant higher TEWL in homozygotes compared to that of ~~and~~ healthy controls¹⁹.

In our study the patient population reacted similarly and therefore no significant difference was detected among the population at either the individual concentrations or at total reactivity by AUC. However, a significant difference was detected compared with the healthy controls.

Regeneration

Only few studies have assessed post-irritation skin regeneration in relation to filaggrin mutations. One study showed a reduction of mRNA expression of profilaggrin in biopsies taken on 1.00% SLS skin sites after 6 hours with succeeding increased regulation²¹. In one clinical study, TEWL measurements were performed to evaluate effect of 1.00% SLS throughout a 72-hour observational period. No significant differences in barrier recovery rate were seen between the groups. Subjects with AD displayed the highest TEWL and vascular response compared with those without AD. Subjects with both AD and filaggrin mutation were not included in this study¹⁸.

In our study regeneration was assessed from 2 different angles: the Day 8 measure and by the factor "R". The Day 8 TEWL measurements were significantly different between those with the atopic phenotype and the healthy controls, and in the clinical assessment between FLGnull_{plus}AD and healthy controls and between FLGnull_{plus}AD and FLGwt_{plus}AD. Full regeneration was not obtained on Day 8 for any patient group (Fig. 4). Our results suggest that regeneration depends on phenotype more than genotype. For regeneration R, no significant difference was found between groups. That the FLGwt_{plus}AD group had the poorest regeneration could be due to a systemic downregulation of filaggrin caused by AD alone, as suggested by others^{35;46}, given that FLGwt_{plus}AD subjects have a functional filaggrin deficiency which induce the same poor barrier as FLGnull_{plus}AD. A plural of studies have been made illustrating filaggrin and AD in univariate analysis; however since filaggrin deficiency is more complex, we applied a multivariate technique. Here we could significantly distinguish the FLGnull_{plus}AD and the FLGwt_{plus}AD from all the remaining groups, showing that these two groups have different reaction patterns (Figure 5). FLGwt_{plus}AD seemed to have the overall highest reactivity driven by component 1 and FLGnull_{plus}AD seemed to be driven by a higher inflammatory alertness, thereby suggesting that this group are early responders (with higher clinical score Day 2, LDF Day 1 and Day 2 and higher TEWL Day 1), which significantly segregates from all the other groups. This segregation was driven by component 2, which primarily depends on LDF Day 1 (Figure 5); however, this segregation was still significant when eliminating the LDF Day 1 from the model. The fact that Δ TEWL in the multivariate model was the driving discriminator, indicates that a high dermal baseline inflammation (LDF Day 1) tends to attenuate the SLS effect as measured by TEWL primarily

for the lowest stimulation doses (SLS 0.25%, 0.50%) whereas when stimulating with SLS 1.00% this protective effect is lost.

This study is the first to examine pattern analysis of skin reaction and regeneration up to 145 hours after removal of Finn chamber with SLS stratified by the filaggrin mutation genotype and AD phenotype. The strength of this study is the population size, compared with that of other clinical studies on pheno-and genotype. It is a limitation that the FLGnull_{non}AD were not healthy individuals, but eczema patients with either irritant contact dermatitis or allergic contact dermatitis. However, we were unable to recruit sufficient individuals with FLG mutations but without skin disease. A control chamber would have been preferred, but was however not applied.

Conclusion

We found that both LDF and TEWL increased markedly in a dose-dependent manner in response to SLS. This suggests a dose-dependent alteration of the skin barrier function, with notable distinction between the patient population and the healthy control group. Moreover, the skin regeneration significantly differed between the patient population with AD and the healthy controls. This study forwards the current knowledge that having filaggrin mutation, AD or both decreases the skin barrier function and regeneration ability. Additionally, those with the AD phenotype, irrespective of filaggrin mutation status, exhibited the greatest response and the poorest regeneration. Subjects with FLGnull_{plus}AD were characterised by multivariate analysis as being more rapid responders primarily driven by having higher inflammatory alertness both compared to the healthy controls, FLGnull_{non}AD and FLGwt_{plus}AD.

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Figure legends

Legend Fig. 1

Categorization of types and distribution of filaggrin mutation and atopic dermatitis (AD). Ntotal = 67 subjects.

The red boxes illustrate those with AD.

¶ Subjects without any of the three most common filaggrin mutations (501X, 2282del4, and 2447X).

† Subjects with one of the three most common filaggrin mutations (501X, 2282del4, and 2447X).

‡ Without AD, according to the UK Working Party's diagnostic criteria for atopic dermatitis.

§ With AD, according to the UK Working Party's diagnostic criteria for atopic dermatitis.

†† Heterozygote for a filaggrin mutation, having filaggrin mutation on one allele.

€ Homozygote for a filaggrin mutation, having filaggrin mutations on both alleles.

Legend Fig. 2

Box Whisker-Plots of baseline laser Doppler flowmetry (LDF), transepidermal water loss (TEWL), pH and skin temperature values, stratified by AD and filaggrin genotype, Ntotal= 67.

Legend Fig.3

Time variation of pH from baseline to Day 8 according to atopic dermatitis (AD) and filaggrin mutations.

Ntotal_{Day1} = 67, Day2 = 66, Day3 = 66 and Day8 = 65.

Legend Fig. 4

a) LDF box Whisker-Plots of the 4 groups at 0.25%, 0.50% and 1.00% SLS over time, stratified by AD and genotype, Ntotal_{Day1} = 67, Day2 = 66, Day3 = 66 and Day8 = 65.

b) TEWL boxplot of the 4 groups at 0.25%, 0.50% and 1.00% SLS over time, stratified by AD and genotype, Ntotal_{Day1} = 67, Day2 = 66, Day3 = 66 and Day8 = 65.

Legend Fig. 5

a) Loading plot from ASCA analysis displaying the most informative pattern in terms of study design. Color and markersize indicate responses (and derived responses) according to day. B) scoreplot of the individual patients distributed across four AD/filaggrin groups (marked by color and markers) for SLS concentrations: B1) 0.25%, B2) 0.50% and B3) 1.00% The ellipses cover 47% (± 1 standard deviation in both directions) of the individual classes.

Table 1. Inclusion, exclusion and restrictions of the study population during the trial

Eligibility criteria	Hospital population, Ntotal= 47 (FLGwt ^{plusAD} , FLGnull ^{nonAD} and FLGnull ^{nonAD})	Healthy controls, Ntotal = 20
Inclusion	Known atopic status, known filaggrin status and age between 18 and 69 years. Caucasian.	Age 18–69 years. Caucasian.
Exclusion	Disease: Active or widespread eczema (but minor eczema was accepted) or chronic inflammatory diseases besides eczema (rheumatoid arthritis, Crohn’s disease, Colitis ulcerosa, Systemic lupus erythromatosis or psoriasis) Treatments: No (UV- treatment of test area within 3 weeks of trial, treatment with topical steroids 2 weeks before trial, application of emollients on the test areas throughout the trial, immune-modulating medical treatment).	No actual or prior eczema. Otherwise both disease and treatment exclusion were as for the hospital population.
Restrictions	No smoking and caffeine consumption within 2 hours before measurements, no consumption of food within 4 hours of measurements, and no analgesics within 24 hours prior to measure.	The same as for the hospital population.

FLGwt are participants without one of the three most common filaggrin mutations (501X, 2282del4, and 2447X). FLGnull are participants with at least one of the three most common filaggrin mutations (501X, 2282del4, and 2447X). ^{nonAD} are participants without atopic dermatitis (AD) but with eczema and ^{plusAD} are with AD according to the UK Working Party’s diagnostic criteria for atopic dermatitis.

Table 2. Information on the non-invasive bioengineering electronic devices and SLS

Manufacturer	Probe name	Performance of measurement
The MPA5 multiprobe adaptor system from Enviroderm, Courage+ Khazaka electronic GmbH, CK electronic	TM300, a preheated probe to measure TEWL	Duration, 30 sec and an average was created from the last 10sec of steady state.
	PH905, measuring pH.	Triplet measurements with 5 sec between measurements, the mean value was used in the analysis
	Skin thermometer probe	Two consecutive skin measurements were performed creating an average.
	A room condition probe	Measuring temperature and humidity continuously.
The laser Doppler flowmetry was a MoorVMS-LDF1 from Moor Instruments Ltd, UK.	MoorVMS-LDF1 single channel 785nm vascular monitoring system with VP1T/7 probe with integrated temperature probe	A mean LDF value was made from 3 consecutive measurements with a steady state for 15 sec. The skin blood flow was measured in arbitrary units (PU).
SLS from Sigma-Aldrich, Steinheim, Germany, 99% purity.		Dilutions in aqueous solution were made, 1%, 0.50% and 0.25% SLS. 50µL SLS was added to a 12mm Finn chamber® on scanpore® tape with filter disc from vitaflo Scandinavia (navamedic AB) ^{9;47} and left for approximately 24 hours upon removal. Measurements were made 1 hour after removal of the chambers ⁴⁸ and all subjects were acclimatized for 15 min in a quiet setting before all measurements.

Table 3. Clarification of the study protocol during the trial

Experimental protocol	
The study period	End October 2011– mid-March 2012.
Randomization of skin sites to SLS	Skin sites on the upper right arm were blindly randomized to an aqueous solution of 0.25%, 0.50% and 1.00% SLS. The first chamber was placed 9 cm from the centre of the axillary fossa and the other chambers in distal extension hereof.
Positioning of the study population	All test subjects were positioned in the same manner with the head of the bed slightly elevated and with the right arm placed behind the head.
Measurement days	The baseline measurements were performed 9 cm distal from the centre of the axillary fossa on non-lesional skin, before irritant application. The measurements were made at baseline before SLS application (Day 1), at 24 hours (Day 2, 1 hour after removal of chamber), 48 hours (Day 3, 25 hours after removal of chamber) and Day 8 after the SLS application (145 hours after removal of Finn chamber).
Forms	- Δ reactions: Here we subtracted the baseline measurements from the Day 2, 3 and 8 measurements, to evaluate the pure reaction caused by SLS. - AUC: This was calculated by i.e. $((\text{medianLDF}_{0.25\% \text{SLS}} + \text{median}_{0.50\% \text{SLS}})/2) \cdot (0.50 - 0.25) + ((\text{medianLDF}_{0.50\% \text{SLS}} + \text{median}_{1.00\% \text{SLS}})/2) \cdot (1.00 - 0.50)$ - Regeneration (R): This was evaluated as follows: if an increase from baseline to max response was Δ_{max} and the difference between baseline and Day 8 was Δ_8 , then R can be expressed in percentage of Δ_{max} : $R = 100 \cdot (\Delta_{\text{max}} - \Delta_8) / \Delta_{\text{max}}$ Thus if R= 100%, the irritated skin will be fully regenerated back to baseline value.
Order of the different measurements and measurement conditions	All visual scorings and instrumental measurements were performed by J. Bandier and in the following order: clinical scoring, skin temperature, TEWL, pH and LDF. Measurements were performed without direct influence of external light sources (blinds drawn and with only soft indirect lighting).

Table 4. Skin reaction to different doses of SLS Day 2

Comparison of skin reactions	0.25% SLS	0.50% SLS	1.00% SLS
Δ reaction LDF	-	Control vs FLGwtplusAD * Control vs FLGnullplusAD ***	Controls vs FLGnullplusAD***
Δ reaction TEWL	-	-	Controls vs FLGwtplusAD*** Controls vs FLGnullplusAD*

Level of significance < 0.05; *p < 0.05; **p < 0.01, ***p < 0.005 in comparison with healthy controls; Ntotal= 66.

Table 5. Reactivity to SLS by Δ AUC on Day 2 and Day 3, evaluated by transepidermal water loss (TEWL) and laser Doppler flowmetry (LDF)

Δ AUC Day 2, Ntotal	TEWL 50% percentile	TEWL Interquartile range	LDF 50% percentile	LDF Interquartile range
Controls	10,3	8.35-13.39	30,31	21.02-52.84
FLGnull _{non} AD	14,00	8.91-16.20	60,02	15.62-101.83
FLGwt _{plus} AD	17,46 ***	11.49-20.50	64,86 *	33.22-94.05
FLGnull _{plus} AD	13,31	10.48-16.77	66,42 ***	47.70-133.27
Δ AUC Day 3, Ntotal				
Controls	18,44	7.08-21.19	34,26	19.50-58.16
FLGnull _{non} AD	18,56	13.63-24.39	58,13	32.72-104.56
FLGwt _{plus} AD	23,40	15.51-32.34	57,48	43.17-69.90
FLGnull _{plus} AD	19,41	11.88-23.98	41,08	30.55-57.72

Level of significance < 0.05; *p < 0.05; **p < 0.01, ***p < 0.005 in comparison with healthy controls.

The results are presented in quartiles. Ntotal_{Day2} and _{Day3} = 66.

Table 6. The regeneration calculated from medians by the formula: $R = 100 \cdot (\Delta_{\max} - \Delta_8) / \Delta_{\max}$

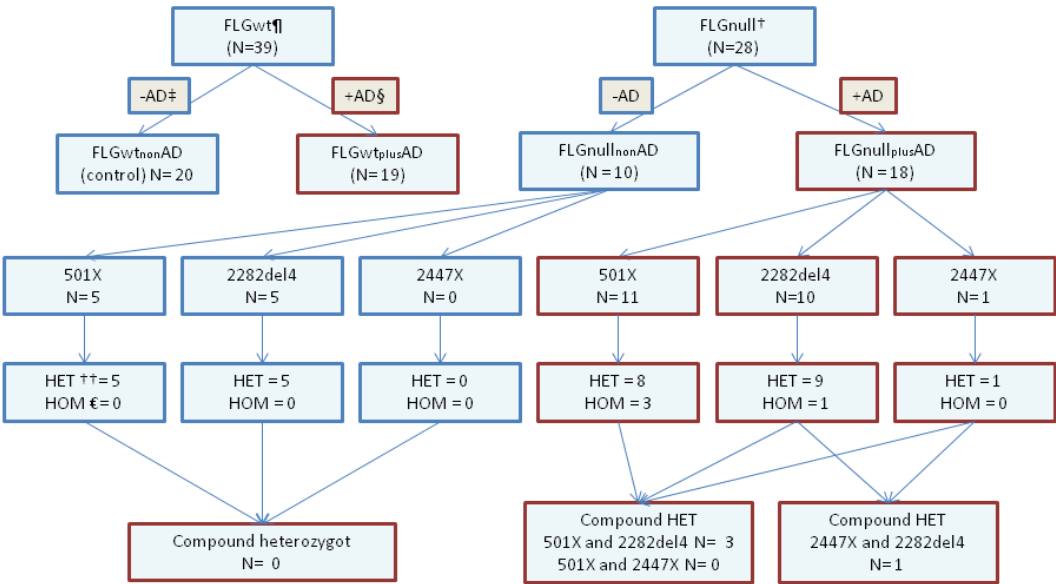
SLS dose %	Healthy controls Ntotal= 19		FLGnull _{non} AD Ntotal= 10		FLGwt _{plus} AD Ntotal= 18		FLGnull _{plus} AD Ntotal= 18	
Quartiles	50% percentile	Quartile range	50% percentile	Quartile range	50% percentile	Quartile range	50% percentile	Quartile range
LDF								
0.25	100.54%	91.58– 122.07	91.11%	84.32– 94.99	90.09%	67.17– 104.33	93.75%	73.33– 101.72
0.50	91.69%	81.27– 103.74	90.62%	78.61– 96.63	81.37%	63.64– 93.43	90.23%	77.13– 100.54
1.00	88.56%	31.37– 95.12	81.41%	65.04– 94.00	60.64%	26.50– 89.11	83.15%	43.18– 85.90
TEWL								
0.25	87.12%	71.89– 96.26	84.87%	66.83– 94.87	79.69%	70.48– 86.96	89.14%	59.13– 115.79
0.50	86.21%	76.67– 94.63	81.96%	76.70– 100.93	70.38%	64.25– 92.77	87.00%	74.78– 101.03
1.00	84.35%	71.48– 89.90	81.20%	72.75– 96.90	75.28%	59.30– 83.72	76.75%	64.83– 83.81

LDF = laser Doppler flowmetry

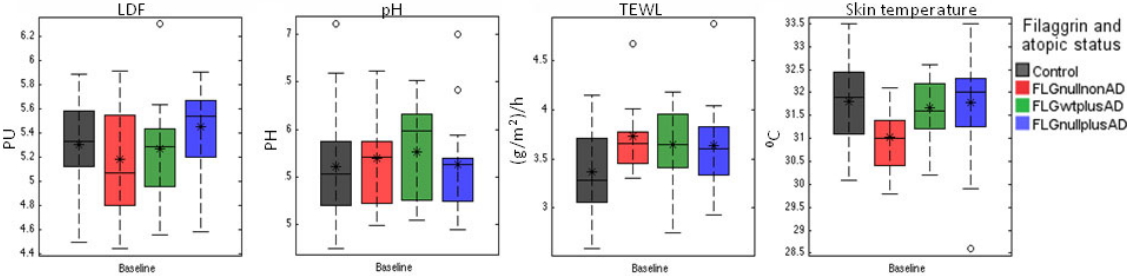
TEWL= Transepidermal Water Loss

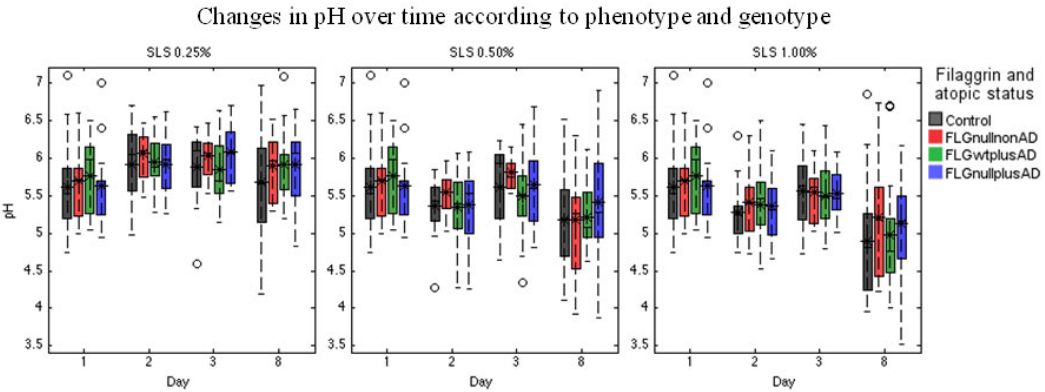
Kruskal-Wallis statistics was used to compare all groups and the individual comparisons were performed by Man-Whitney statistics. The results are presented in quartiles. Regeneration R is presented as a percentage of $\Delta_{\max}$. Ntotal= 65.

Distribution of the atopic dermatitis phenotype and filaggrin genotype

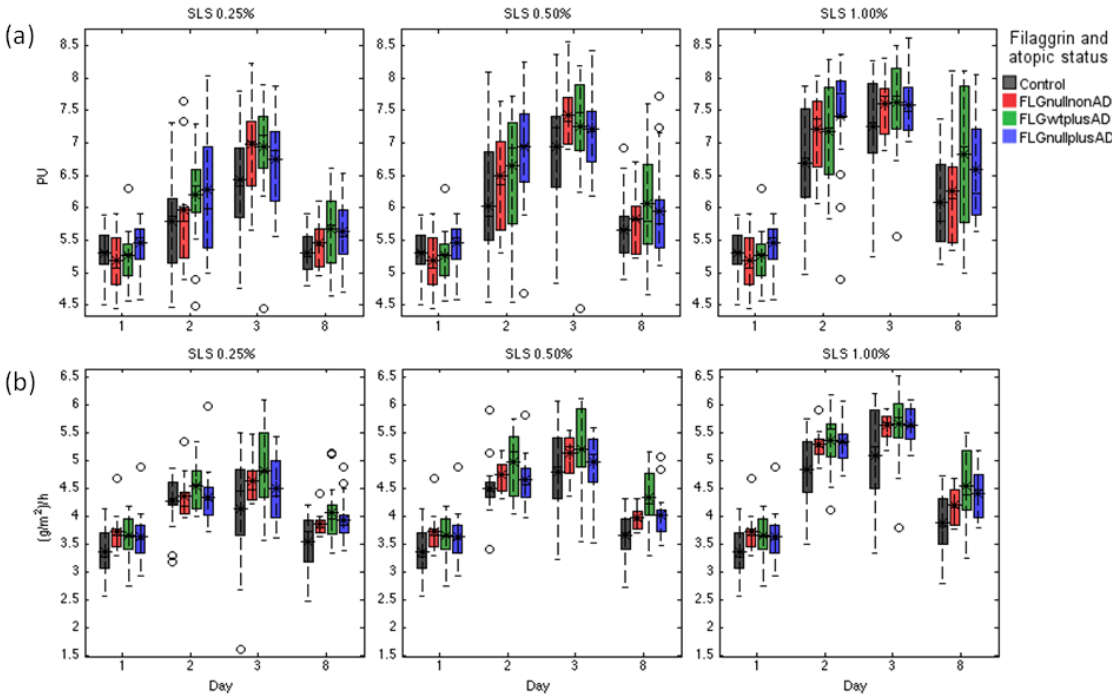


Baseline values (day1)





Measurement of laser Doppler flowmetry and TEWL according to filaggrin genotype and atopic status



Variation in baseline and SLS responses according to filaggrin genotype and atopic status

