**Different types of emollient cream exhibit diverse physiological effects on the skin barrier in adults with atopic dermatitis**

Supporting Information

# Supplementary methods

## Participants

A single cohort of volunteers was recruited at the Sheffield Dermatology Research, Skin Barrier Research Facility from Feb 2019 until June 2019. Recruitment was open to male and female volunteers aged 18 years or above, who were recruited on a first come first served basis, assuming they met the specified criteria. Inclusion criteria included having a self-reported recent (with the past 12 months) history of AD defined by the UK working party diagnostic criteria.1

Exclusion criteria included: eczema on the volar forearms requiring anti-inflammatory treatment; possible allergy to ingredients in the study medications; any serious current medical condition which, in the opinion of the Investigator, may interfere with the evaluation of the results or may be contraindicated by the use of the test medications; use of any concomitant medication that may interfere with the study related activities or assessment of efficacy, as judged by the Investigator; use of any topical product, including cosmetic leave-on products on the volar forearms, within 1 week prior to, and throughout the study; female participant who, according to the participant, is pregnant or breast-feeding, or plans to become pregnant during the course of the study; any participant-related factor suggesting potential poor compliance with study procedures (e.g. psychiatric disorders, history of alcohol or substance abuse), as judged by the Investigator; and enrolment in any interventional study or use of an investigational drug within 3 months prior to the screening visit.

During the screening visit demographic information was collected and an assessment of AD severity was made by a dermatologist using the investigator global assessment of severity and the Nottingham eczema severity score (because this gives an indication of eczema severity over the last 12 months).2

Informed consent was obtained from each participant. All participants received remuneration appropriate for their involvement.

## Randomization and masking

Treatment allocation to the four treatment areas was randomized and balanced. Table S1 provides details of the allocation of each treatment to each area of skin. The randomization schedule was created by the Statistical Services Unit, University of Sheffield, using a block randomisation scheme. The study was conducted observer-blind; product identities were concealed from the investigators and participants (the no treatment control could not be concealed from the participants).

To maintain blinding all investigational products were supplied in plain packaging with blinded research labels, identifying the products using only a code and colour (to minimize the risk of mix-up), by Pharmavize, Belgium. TC and Glycerol cream were produced for this study in research packaging, whereas Paraffin cream was outsourced and provided in manufacturer packaging covered with concealing labels. The identities of the treatment codes were known only to Pharmavize until after database lock. Owing to the different properties of the investigational products (appearance, consistency and size of tube), all treatment related activities were conducted by study team members who were not involved in the collection of the study outcomes, except for treatment consumption, and participants were asked not to discuss the properties of the investigational products with the investigators.

## Treatment and compliance

All participants undertook all 4 treatment conditions. Each forearm (volar face) was divided into 2 areas, providing 4 possible treatment areas per subject. Each area was approximately 140cm2 (equal to 1 hand3). To avoid cross-contamination participants were asked not to spread the treatments too close together at the junction between the two areas on each arm. No measurements were taken at or near the junction (4cm clearance minimum). Participants received training on how to dose and apply the products, and undertook 3 supervised applications. Treatment compliance was assessed after 5, 15 and 29 days based on completion of treatment dairies (logs capturing the time of each application) and treatment weights. To be compliant with the protocol participants were required to apply the products on >75% of the treatment days and apply an average of 1g ±0.5g per day, which equates to about 7 ±3.5 mg/cm2/day.

## Biophysical measurements

Skin redness, related to erythema, was measured using a colourimeter (the Mexameter MX18, CK electronic GmbH, Cologne, Germany). Skin hydration/moisturization was measured indirectly as electrical capacitance (high capacitance corresponds to high stratum corneum water content) using a Corneometer CM825 (CK electronic GmbH, Cologne, Germany). Skin barrier function was determined indirectly by measuring Transepidermal Water Loss (TEWL) from the skin surface (a measure of inside to outside water permeability) using an AquaFlux AF200 condensing chamber probe (Biox Systems Ltd., London, UK). Each of the aforementioned biophysical measurements was taken in sequence as written and in triplicate (3 repeats), collected from adjacent locations within each treatment area. Images of the skin sites were captured at 50x magnification using the c-cube digital dermoscope (Pixience, Toulouse, France), from which the erythema index (2D colour analysis) and skin surface dryness (3D topographical analysis) was calculated. All assessments were performed in a room maintained at 21±2°C and 38-50% relative humidity according to published guidelines.4 All test sites were acclimatised to room conditions for 20 minutes before assessment.

## Skin sensitivity testing

Between 12 and 18 hours after the last application of product, two sites within each treatment area (including the untreated control area) were covered with 12mm Finn chambers on Scanpor tape (Smart Practice, Phoenix, USA) containing 50µl 1.0% sodium lauryl sulfate (SLS) prepared in deionized water on a filter disc insert (Whatman, Maidstone, UK). The chambers were then covered with PatchProtect (Smart Practice) water resistant adhesive dressings and left in place for 24h, before being carefully removed by the participant at home. Visual grading of erythema and objective assessment of irritation based of TEWL, redness (Mexameter) and Erythema Index (c-cube) measurements, was performed before patch application and again 24h following patch removal.

## Tolerability

The participants were asked to score the degree of smarting/burning sensation (a sharp, local,

superficial effect which can be experienced during contact with for example acidic solutions)

using a VAS scale, at the start of treatment, after 5 days and again after 4 weeks of treatment. Visual Redness was graded on a 4-point scale from 0 to 3, where 0 is no erythema/reaction and 3 is strong/marked erythema/reaction.

## Quantification of NMF constituents in the SC

NMF is a collection of natural hydroscopic compounds responsible for maintaining skin moisture, including sodium pyrrolidine carboxylic acid, urocanic acid, and free amino acids analysed here. Urea is also a natural component of NMF, however we have restricted our analysis to components not in the treatments so that the findings correspond to changes in endogenously produced NMF. Adapted from a published assay5, SC collected by tape stripping (discs 4-6) was cut and pooled in 750µl methanol. Samples were then subjected to an ultrasonic bath, followed by agitation at 4°C (20 mins each), filtered using a 0.2µm syringe filter and dried. Distilled water (120µl) was used to resuspend samples before analysis. Isocratic elution of sodium pyrrolidine carboxylic acid (210nm) and urocanic acid (270nm) was performed in 0.1M phosphate buffer (pH 2.75) containing 1% acetonitrile using a Shimadzu HPLC system (Shimadzu, Kyoto, Japan) equipped with Synergi Hydro RP column (Phenomenex, Macclesfield, UK). 25µl of sample was injected in duplicate. The same extract was used to quantify free amino acids by o-phthalaldehyde derivatization.6 Quantification of each NMF component was achieved by standard curve interpolation and normalised relative to the amount of SC removed by tape stripping.

## *FLG* genotyping

Genomic DNA was extracted from Buccal swabs collected during the first study visit using the QIAamp DNA mini kit (Qiagen, Hilden, Germany). Screening of the 4 common European mutations was achieved by Taqman probes (R501X and 2282del4) and sequencing (R2447X and S3247X) using established primer / probe and 7900HT / 3730 instruments (Applied Biosystems, California, USA).7 Samples were not collected from 1 participant and the full *FLG* genotypes of two samples could not be determined, providing 46 participant samples in total.

## Role of the funding source

The funder was also the trial Governance Sponsor. The Sheffield Dermatology group designed and conducted the study. The analysis was undertaken by the Sheffield Statistical Services Unit. The funder was involved in the design and monitored the conduct of the study from a quality assurance perspective, but was not directly involved in the delivery of the study at the site or in the analysis of the study data.

## Statistical analysis

The total consumption of the creams (test or reference cream), based on cream weight was calculated and tabulated descriptively.

The statistical and analytical plan (SAP), dated 04 November 2019, was prepared and approved before database lock. Database lock was 29 November 2019. Analyses were done with SAS version 9.4.

# Supplementary results

All 49 participants received the treatments, which were randomly allocated using a balanced scheme to minimise site dependent effects, and completed the study. Protocol deviations occurred in 13 participants and comprised: use of restricted medications (5 participants); average daily use of the product exceeding 1.5g (4 participants), one or more patches coming off early (3 participants), visits occurring outside the protocol window (2 participants), and treatment stopped at one site early (1 participant). In the last case, treatment with Paraffin cream was terminated after 16 days due to an adverse event. Only product use exceeding 1.5g per day on average was considered a major protocol deviation that could affect the study results. All 49 participants completing the study were included in the full analysis set (FAS). A per protocol analysis set (PPS) excluding participants with major protocol deviations was performed and yielded consistent results with the FAS.

Despite the average use of >1.5g of cream per day by 4 participants (no participants applied <0.5g), cream usage was very consistent between the different products and follows the density of the products, with TC being the densest and Paraffin cream being the least dense.

All skin assessments were conducted in a climate-controlled environment, with a temperature of 21.3±0.91 (range 19.1-23.4) oC and relative humidity of 45.0±2.41 (range 40.1-51.2) %.

## Erythema index taken from 2D skin images provides a robust objective measure for skin redness

Skin redness following SLS-challenge was measured objectively using the Mexameter, a previously validated method that can be used to predict visual skin redness (Figure S2).8 A change in 47.67 Mexameter units roughly corresponds to a change in visual redness of 1 point (mexameter\_change = 38.56 + 47.67\*visual\_red, r2 = 0.49). As a secondary outcome (due to novelty) skin redness was also determined by quantifying the red colour in the colour calibrated 2D dermoscope images. The Erythema Index positively associated with Mexameter measurement of redness as expected (Spearman’s r 0.628) and was similarly predictive (Figure S3). For reference a change of approximately 5.80 Erythema Index units (EIU) equated to a 1-point change on the visual scale (eryth\_change = 9.15 + 5.80\*visual\_red, r2 = 0.44).

## Tolerability

More than three quarters of patients gave a VAS score of 0, therefore after a review of this data it was agreed that aside from a table of summary statistics (Table S5) no analysis would be carried out. Visual redness was scored at 0, on a 4-point scale from 0-3, after cessation of treatment (day 29) in all participants, and so no analysis was performed. Objective determination of skin erythema from 2D color skin images (erythema index) after 4 weeks treatment showed that there was no significant difference between TC and NTC (estimated difference -0.526, 95% CI (-1.261, 0.209), p=0.160) or TC and Paraffin cream (estimated difference -0.239, 95% CI (-0.981, 0.502), p=0.525) (tables S4 and S6). There was a slight increased erythema in skin treated with Glycerol cream, which made the difference between TC and Glycerol cream significant (estimated difference -0.739, 95% CI (-1.528, -0.057), p=0.035), however, this slight increase, which was not observable by eye, was not considered clinically relevant.

The most common adverse events (Table S7) were eczema (TC, 2%; Glycerol cream, 4%; and Paraffin cream, 2%), application site erythema (TC, 0%; Glycerol cream, 2%; and Paraffin cream, 6%), and application site rash (TC, 0%; Glycerol cream, 0%; and Paraffin cream, 6%).

# Supplementary discussion

After 4 weeks treatment but prior to SLS-challenge, the reduction in basal TEWL induced by all treatments was marginally (below a clinically relevant level), but significantly, less than that exhibited at untreated sites. The relationship between degree of skin dryness and TEWL is complex and elevated TEWL levels can be found both in hyperhydrated and in dry skin9. In humans, there is a continuous diffusion of water from within the body, through the SC and into the environment as a result of a natural water concentration gradient. This gradient varies as the relative humidity of the environment changes, the structure of the skin changes and as the water concentration of the SC increases10. As the relative humidity increases one might expect a lower flux because the gradient decreases as a result of the increased water concentration in the outside cell layers. As the water concentration of the tissue increases, however, diffusing water molecules encounter lower restraining forces (higher diffusion constants) and for this reason the flux might increase. TC and Glycerol cream both increased the amount of bound water in the skin, as shown by increased skin capacitance, and so the observed change in TEWL cannot be interpreted as an effect solely on skin barrier function. On the other hand, Paraffin cream, which did not increase the amount of bound water in the skin, displayed increased TEWL relative to the NTC and imparted no protection from SLS. Imperceptible residues left on the skin surface (last application was made the night before measurements were taken) may also interfere with the measurements taken, by increasing surface water binding or reducing TEWL.

Leung and colleagues reported that the extent of skin barrier dysfunction assessed using the skin tape-stripping procedure correlated with food-specific IgE and the number of positive skin tests for food allergens, suggesting that topical products could modify the risk of developing allergy by affecting the skin barrier.11 Paraffin cream was one of the interventions in a recent definitive eczema prevention clinical trial that failed to demonstrate a protective effect of daily moisturizer use from birth.12,13 Moreover, there was a trend for increased food allergy (not significant) in the intervention group, raising the question of whether the negative effects of emollients like Paraffin cream result in increased susceptibility to allergens as well as irritants. Other eczema-prevention studies, albeit of smaller scale, have reported reduced rates of AD when using different moisturizer interventions.14,15 The results of this study highlight the differences in effects of different moisturizers on the skin barrier, providing an explanation for why these eczema prevention trials may have produced differing results.

# Supplementary Figures

**Figure S1:** Treatment with TC is well tolerated and reduces skin erythema in response to SLS exposure. (A) Representative colour images (16x12mm) of the volar forearm, acquired before treatment (Day 1), during (Day 15) and after the treatment period (29) and again 24hrs after removal of the SLS patches (Day 31). (B) Image rendering highlights areas of erythema.



**Figure S2:** Relationship between Mexameter units and visual skin redness. The lines indicate the 95% prediction interval.



**Figure S3:** Relationship between erythema index and visual skin redness. The lines indicate the 95% prediction interval.



# Supplementary Tables

**Table S1:** Treatment allocation frequencies to the test sites

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| ***Treatment condition*** | ***Upper left forearm*** | ***Lower left forearm*** | ***Upper right forearm*** | ***Lower right forearm*** |
| TC | 12 | 12 | 13 | 12 |
| Glycerol cream | 12 | 12 | 13 | 12 |
| Paraffin cream | 13 | 12 | 12 | 12 |
| No treatment | 12 | 13 | 11 | 13 |

**Table S2:** Summary statistics for the primary outcome measures

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| ***Statistic*** | ***Test cream*** | ***Glycerol cream*** | ***Paraffin cream*** | ***Untreated control*** |
| *Visual redness score at day 31:* |
| *n* | 48 | 48 | 47 | 48 |
| Mean | 0.99 | 1.08 | 1.45 | 1.35 |
| StDev | 0.691 | 0.649 | 0.718 | 0.680 |
| Median | 1.00 | 1.00 | 1.50 | 1.25 |
| Min | 0.0 | 0.0 | 0.0 | 0.0 |
| Max | 3.0 | 2.5 | 3.0 | 2.5 |
| *Objective redness based on Mexameter (MU), change from day 29 to day 31:* |
| *n* | 48 | 48 | 47 | 48 |
| Mean | 80.859 | 82.893 | 106.39 | 98.951 |
| StDev | 53.901 | 53.150 | 56.315 | 53.917 |
| Median | 79.500 | 87.750 | 107.00 | 107.69 |
| Min | -8.500 | -29.13 | -29.00 | -0.500 |
| Max | 217.63 | 188.00 | 236.75 | 223.63 |
| *Objective redness based on Erythema index (EIU), change from day 29 to day 31:* |
| *n* | 46 | 46 | 45 | 46 |
| Mean | 13.261 | 14.833 | 17.981 | 16.747 |
| StDev | 6.9086 | 6.0440 | 7.3550 | 6.7629 |
| Median | 11.631 | 13.913 | 18.972 | 15.342 |
| Min | 1.816 | 2.740 | 1.976 | 3.430 |
| Max | 32.980 | 33.184 | 35.379 | 35.393 |
| *TEWL (g/m2/h), change from day 29 to day 31:* |
| *n* | 48 | 48 | 47 | 48 |
| Mean | 19.790 | 24.680 | 29.648 | 28.646 |
| StDev | 13.860 | 14.693 | 14.299 | 13.668 |
| Median | 15.245 | 21.278 | 27.266 | 25.496 |
| Min | 4.550 | 3.223 | 4.681 | 8.035 |
| Max | 66.069 | 64.578 | 74.918 | 64.248 |

**Table S3:** Summary of primary outcome measure of skin sensitivity for the PPS

|  |  |  |  |
| --- | --- | --- | --- |
| ***Comparison*** | ***Estimated Difference*** | ***95% Confidence Interval*** | ***p-value*** |
| *TEWL (g/m2/h), change from day 29 to day 31: a* |
| TC vs NTC | -10.874 | -14.115, -7.632 | **<0.001** |
| TC vs Glycerol cream | -4.141 | -7.412, -0.871 | **0.013** |
| TC vs Paraffin cream | -11.224 | -14.493, -7.955 | **<0.001** |

*a*, ANCOVA model includes fixed factor for treatment, random effect for patient and covariate for day 29 measure

**Table S4:** Summary of secondary outcome measures

|  |  |  |  |
| --- | --- | --- | --- |
| ***Comparison*** | ***Estimated Difference*** | ***95% Confidence Interval*** | ***p-value*** |
| *TEWL (g/m2/h), change from day 1 to day 29: a* |
| TC vs NTC | 0.514 | 0.086, 0.943 | **0.019** |
| TC vs Glycerol cream | -0.223 | -0.650, 0.205 | 0.305 |
| TC vs Paraffin cream | -0.093 | -0.525, 0.338 | 0.670 |
|  Glycerol cream vs NTC *b* | 0.737 | 0.311, 1.163 | **<0.001** |
| Paraffin creamvs NTC *b* | 0.607 | 0.179, 1.036 | **0.006** |
| *Capacitance (AU), change from day 1 to day 29:  a* |
| TC vs NTC | 5.739 | 3.767, 7.711 | **<0.001** |
| TC vs Glycerol cream | -0.783 | -2.753, 1.188 | 0.434 |
| TC vs Paraffin cream | 5.303 | 3.311, 7.294 | **<0.001** |
| *Skin surface dryness (Sa[µm]), change from day 1 to day 29: a* |
| TC vs NTC | -0.894 | -1.760, -0.029 | **0.043** |
| TC vs Glycerol cream | -0.069 | -0.931, 0.793 | 0.874 |
| TC vs Paraffin cream | -1.152 | -2.023, -0.281 | **0.010** |
| *NMF levels (mmol/g protein), at day 29: c* |
| TC vs NTC | 0.394 | 0.180, 0.609 | **<0.001** |
| TC vs Glycerol cream | 0.118 | -0.095, 0.331 | 0.275 |
| TC vs Paraffin cream | 0.850 | 0.636, 1.065 | **<0.001** |
| *Erythema index (AU), change from day 1 to day 29: a* |
| TC vs NTC | -0.526 | -1.261, 0.209 | 0.160 |
| TC vs Glycerol cream | -0.793 | -1.523, -0.057 | **0.035** |
| TC vs Paraffin cream | -0.239 | -0.981, 0.502 | 0.523 |

*a*, ANCOVA model includes fixed factor for treatment, random effect for patient and covariate for day 1 measure

*b*, Post-hoc analysis

*c*, ANCOVA model includes fixed factor for treatment, and random effect for patient

**Table S5:** Summary of visual analogue scores for tolerability

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| ***Day*** | ***Statistic***  | ***Test cream*** | ***Glycerol cream*** | ***Paraffin cream*** |
| 1 | n | 49 | 49 | 49 |
|   | Mean | 0.16 | 0.14 | 0.57 |
|   | StDev | 0.408 | 0.357 | 1.392 |
|  | Median | 0.00 | 0.00 | 0.00 |
|   | Min | 0.0 | 0.0 | 0.0 |
|   | Max | 2.0 | 1.6 | 6.2 |
| 15 | n | 49 | 49 | 49 |
|  | Mean | 0.14 | 0.14 | 0.18 |
|  | StDev | 0.402 | 0.464 | 0.541 |
|  | Median | 0.00 | 0.00 | 0.00 |
|  | Min | 0.0 | 0.0 | 0.0 |
|   | Max | 1.8 | 2.6 | 2.9 |
| 29 | n | 48 | 48 | 48 |
|  | Mean | 0.08 | 0.11 | 0.08 |
|   | StDev | 0.324 | 0.313 | 0.300 |
|  | Median | 0.00 | 0.00 | 0.00 |
|  | Min | 0.0 | 0.0 | 0.0 |
|  | Max | 2.1 | 1.6 | 1.9 |

**Table S6:** Summary of erythema index change from day 1 to day 29

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Statistic**  | **Test cream** | **Glycerol cream** | **Paraffin cream** | **Untreated control** |
| *n* | *48* | *48* | *47* | *48* |
| Mean | -0.047 | 0.721 | -0.016 | 0.508 |
| StDev | 4.2043 | 4.5420 | 4.8371 | 3.7738 |
| Median | 0.025 | 0.334 | 0.740 | -0.046 |
| Min | -9.938 | -8.823 | -13.78 | -6.319 |
| Max | 9.176 | 10.326 | 9.394 | 8.792 |

**Table S7:** Adverse events (patient level) by system organ class and preferred term

|  |  |  |  |
| --- | --- | --- | --- |
| **System Organ Class** | **Preferred Term** | **No single treatment identified** | **Where single treatment is identified** |
| *Test cream* | *Glycerol cream* | *Paraffin cream* |
| *Infections and infestations* | All patients | 11 (22%) | 0 | 0 | 0 |
|  | Cystitis | 1 (2%) | 0 | 0 | 0 |
|  | Gastrointestinal infection | 1 (2%) | 0 | 0 | 0 |
|  | Lower respiratory tract infection | 1 (2%) | 0 | 0 | 0 |
|  | Nasopharyngitis | 8 (16%) | 0 | 0 | 0 |
| *Neoplasms benign, malignant and unspecified (incl cysts and polyps)* | All patients | 0 | 0 | 0 | 1 (2%) |
| Melanocytic naevus | 0 | 0 | 0 | 1 (2%) |
| *Immune system disorders* | All patients | 2 (4%) | 0 | 0 | 0 |
|  | Seasonal allergy | 2 (4%) | 0 | 0 | 0 |
| *Nervous system disorders* | All patients | 12 (24%) | 0 | 0 | 0 |
|  | Headache | 9 (18%) | 0 | 0 | 0 |
|  | Migraine | 1 (2%) | 0 | 0 | 0 |
|  | Paraesthesia | 2 (4%) | 0 | 0 | 0 |
| *Eye disorders* | All patients | 1 (2%) | 0 | 0 | 0 |
|  | Eye pain | 1 (2%) | 0 | 0 | 0 |
| *Respiratory, thoracic and mediastinal disorders* | All patients | 7 (14%) | 0 | 0 | 0 |
| Cough | 3 (6%) | 0 | 0 | 0 |
|  | Epistaxis | 1 (2%) | 0 | 0 | 0 |
|  | Oropharyngeal pain | 3 (6%) | 0 | 0 | 0 |
|  | Sneezing | 1 (2%) | 0 | 0 | 0 |
| *Skin and subcutaneous tissue disorders* | All patients | 12 (24%) | 1 (2%) | 2 (4%) | 2 (4%) |
| Dry skin | 1 (2%) | 0 | 0 | 0 |
|  | Eczema | 11 (22%) | 1 (2%) | 2 (4%) | 1 (2%) |
|  | Rash erythematous | 1 (2%) | 0 | 0 | 0 |
|  | Rash macular | 0 | 0 | 0 | 1 (2%) |
|  | Skin fissures | 1 (2%) | 0 | 0 | 0 |
| *Musculoskeletal and connective tissue disorders* | All patients | 5 (10%) | 0 | 0 | 0 |
| Back pain | 2 (4%) | 0 | 0 | 0 |
| Costochondritis | 1 (2%) | 0 | 0 | 0 |
| Musculoskeletal pain | 1 (2%) | 0 | 0 | 0 |
| Pain in extremity | 1 (2%) | 0 | 0 | 0 |
| *Reproductive system and breast disorders* | All patients | 3 (6%) | 0 | 0 | 0 |
| Dysmenorrhoea | 2 (4%) | 0 | 0 | 0 |
| Endometriosis | 1 (2%) | 0 | 0 | 0 |
| *General disorders and administration site conditions* | All patients | 9 (18%) | 0 | 3 (6%) | 9 (18%) |
| Application site bruise | 2 (4%) | 0 | 0 | 2 (4%) |
| Application site eczema | 0 | 0 | 0 | 1 (2%) |
| Application site erythema | 1 (2%) | 0 | 1 (2%) | 3 (6%) |
| Application site irritation | 1 (2%) | 0 | 0 | 0 |
| Application site pain | 1 (2%) | 0 | 0 | 0 |
| Application site papules | 0 | 0 | 2 (4%) | 0 |
| Application site pruritus | 2 (4%) | 0 | 0 | 2 (4%) |
| Application site rash | 2 (4%) | 0 | 0 | 3 (6%) |
| Application site reaction | 0 | 0 | 0 | 1 (2%) |
| Application site urticaria | 0 | 0 | 1 (2%) | 0 |
| Application site vesicles | 1 (2%) | 0 | 0 | 0 |
| Application site warmth | 1 (2%) | 0 | 0 | 0 |
| Asthenia | 1 (2%) | 0 | 0 | 0 |
| Fatigue | 2 (4%) | 0 | 0 | 0 |
| Pain | 1 (2%) | 0 | 0 | 0 |
| *Injury, poisoning and procedural complications* | All patients | 4 (8%) | 0 | 0 | 0 |
| Accident | 1 (2%) | 0 | 0 | 0 |
| Arthropod bite | 1 (2%) | 0 | 0 | 0 |
| Contusion | 1 (2%) | 0 | 0 | 0 |
| Road traffic accident | 1 (2%) | 0 | 0 | 0 |
| Scratch | 1 (2%) | 0 | 0 | 0 |
| Upper limb fracture | 1 (2%) | 0 | 0 | 0 |

**Table S8:** Summary of TEWL (g/m2/h) by *FLG* genotype

|  | **No mutation** | **FLG mutation** |
| --- | --- | --- |
| **Visit** | **Statistic**  | *Test cream* | *Glycerol cream* | *Paraffin cream* | *Untreated control* | *Test cream* | *Glycerol cream* | *Paraffin cream* | *Untreated control* |
| 1 | *n* | 35 | 35 | 35 | 35 | 11 | 11 | 11 | 11 |
|  | *Mean* | 10.514 | 10.898 | 10.934 | 10.924 | 10.491 | 11.801 | 11.801 | 11.836 |
|  | *StDev* | 2.5450 | 2.9343 | 3.2656 | 3.4862 | 1.9788 | 4.3958 | 3.0641 | 2.1146 |
|  | *Median* | 9.867 | 10.108 | 9.762 | 10.110 | 9.601 | 10.870 | 10.738 | 12.040 |
|  | *Min* | 5.655 | 7.065 | 6.775 | 5.800 | 8.963 | 8.995 | 9.197 | 9.280 |
|  | *Max* | 17.542 | 17.822 | 23.483 | 22.936 | 15.421 | 24.613 | 19.744 | 16.120 |
| 29 | *n* | 35 | 35 | 34 | 35 | 11 | 11 | 11 | 11 |
|  | *Mean* | 10.532 | 11.009 | 10.910 | 10.152 | 9.661 | 10.773 | 10.549 | 10.387 |
|  | *StDev* | 2.0794 | 2.1828 | 2.7720 | 3.0404 | 2.3292 | 3.0594 | 2.8525 | 2.2021 |
|  | *Median* | 10.405 | 10.868 | 10.177 | 9.363 | 9.352 | 10.341 | 8.972 | 9.543 |
|  | *Min* | 6.237 | 7.226 | 6.780 | 5.495 | 7.397 | 7.318 | 7.802 | 8.024 |
|  | *Max* | 15.972 | 16.739 | 19.705 | 20.391 | 14.785 | 18.770 | 15.930 | 14.545 |
| 31 | *n* | 35 | 35 | 34 | 35 | 11 | 11 | 11 | 11 |
|  | *Mean* | 31.004 | 34.953 | 40.073 | 38.728 | 25.182 | 35.938 | 44.426 | 41.787 |
|  | *StDev* | 15.306 | 13.842 | 14.543 | 15.543 | 8.5091 | 18.490 | 18.092 | 12.788 |
|  | *Median* | 26.728 | 31.354 | 37.018 | 33.880 | 22.557 | 29.033 | 43.766 | 40.269 |
|  | *Min* | 11.837 | 19.465 | 18.868 | 17.303 | 12.499 | 13.545 | 18.985 | 26.204 |
|  | *Max* | 77.676 | 81.316 | 84.943 | 75.267 | 41.854 | 77.545 | 76.085 | 71.975 |

**Table S9:** Summary of SC NMF levels on day 29 by *FLG* genotype

|  | **No mutation** | **FLG mutation** |
| --- | --- | --- |
| **Statistic**  | *Test cream* | *Glycerol cream* | *Paraffin cream* | *Untreated Control* | *Test cream* | *Glycerol cream* | *Paraffin cream* | *Untreated Control* |
| *n* | 35 | 35 | 34 | 34 | 11 | 11 | 11 | 11 |
| *Mean* | 2.93 | 2.82 | 2.01 | 2.55 | 1.79 | 1.72 | 1.16 | 1.39 |
| *StDev* | 0.963 | 0.997 | 0.578 | 0.799 | 0.765 | 0.806 | 0.527 | 0.523 |
| *Median* | 2.99 | 2.92 | 2.02 | 2.50 | 1.79 | 1.56 | 0.96 | 1.28 |
| *Min* | 1.2 | 0.8 | 1.1 | 0.8 | 0.8 | 0.7 | 0.3 | 0.7 |
| *Max* | 5.0 | 4.7 | 3.5 | 4.4 | 2.8 | 3.0 | 1.8 | 2.4 |

# Supplementary References

1 Williams H, Burney P, Hay R, Archer C, Shipley M, Hunter J. The UK working party’s diagnostic criteria for atopic dermatitis. I. Derivation of a minimum set of discriminators for atopic dermatitis. *Br J Dermatol* 1994; **131**.

2 Emerson RM, Charman CR, Williams HC. The Nottingham Eczema Severity Score: preliminary refinement of the Rajka and Langeland grading. *Br J Dermatol* 2000; **142**.

3 Long CC, Finlay AY, Averill RW. The Rule of Hand: 4 Hand Areas=2 FTU=1 g. *Archives of Dermatology* 1992; **128**: 1129-30.

4 Pinnagoda J, Tupker RA, Agner T, Serup J. Guidelines for transepidermal water loss (TEWL) measurement. A report from the Standardization Group of the European Society of Contact Dermatitis. *Contact Dermatitis* 1990; **22**: 164-78.

5 Takada S, Naito S, Sonoda J, Miyauchi Y. Noninvasive In Vivo Measurement of Natural Moisturizing Factor Content in Stratum Corneum of Human Skin by Attenuated Total Reflection Infrared Spectroscopy. *Applied Spectroscopy* 2012; **66**: 26-32.

6 Nakagawa N, Sakai S, Matsumoto M, Yamada K, Nagano M, Yuki T, Sumida Y, Uchiwa H. Relationship between NMF (lactate and potassium) content and the physical properties of the stratum corneum in healthy subjects. *J Invest Dermatol* 2004; **122**: 755-63.

7 Sandilands A, Terron-Kwiatkowski A, Hull PR, O'Regan GM, Clayton TH, Watson RM, Carrick T, Evans AT, Liao H, Zhao Y, Campbell LE, Schmuth M, Gruber R, Janecke AR, Elias PM, van Steensel MA, Nagtzaam I, van Geel M, Steijlen PM, Munro CS, Bradley DG, Palmer CN, Smith FJ, McLean WH, Irvine AD. Comprehensive analysis of the gene encoding filaggrin uncovers prevalent and rare mutations in ichthyosis vulgaris and atopic eczema. *Nat Genet* 2007; **39**: 650-4.

8 Leskur D, Bukić J, Petrić A, Zekan L, Rušić D, Šešelja Perišin A, Petrić I, Stipić M, Puizina-Ivić N, Modun D. Anatomical site differences of sodium lauryl sulfate-induced irritation: randomized controlled trial. *Br J Dermatol* 2019; **181**: 175-85.

9 Loden M, Andersson AC, Andersson C, Frodin T, Oman H, Lindberg M. Instrumental and dermatologist evaluation of the effect of glycerine and urea on dry skin in atopic dermatitis. *Skin Res Technol* 2001; **7**: 209-13.

10 Blank IH, Moloney J, 3rd, Emslie AG, Simon I, Apt C. The diffusion of water across the stratum corneum as a function of its water content. *J Invest Dermatol* 1984; **82**: 188-94.

11 Leung DYM, Calatroni A, Zaramela LS, LeBeau PK, Dyjack N, Brar K, David G, Johnson K, Leung S, Ramirez-Gama M, Liang B, Rios C, Montgomery MT, Richers BN, Hall CF, Norquest KA, Jung J, Bronova I, Kreimer S, Conover Talbot C, Crumrine D, Cole RN, Elias P, Zengler K, Seibold MA, Berdyshev E, Goleva E. The nonlesional skin surface distinguishes atopic dermatitis with food allergy as a unique endotype. *Sci Transl Med* 2019; **11**: eaav2685.

12 Danby SG, Chalmers J, Brown K, Williams HC, Cork MJ. A functional mechanistic study of the effect of emollients on the structure and function of the skin barrier. *Br J Dermatol* 2016; **175**: 1011-9.

13 Chalmers JR, Haines RH, Bradshaw LE, Montgomery AA, Thomas KS, Brown SJ, Ridd MJ, Lawton S, Simpson EL, Cork MJ, Sach TH, Flohr C, Mitchell EJ, Swinden R, Tarr S, Davies-Jones S, Jay N, Kelleher MM, Perkin MR, Boyle RJ, Williams HC. Daily emollient during infancy for prevention of eczema: the BEEP randomised controlled trial. *Lancet* 2020; **395**: 962-72.

14 Horimukai K, Morita K, Narita M, Kondo M, Kitazawa H, Nozaki M, Shigematsu Y, Yoshida K, Niizeki H, Motomura K-i, Sago H, Takimoto T, Inoue E, Kamemura N, Kido H, Hisatsune J, Sugai M, Murota H, Katayama I, Sasaki T, Amagai M, Morita H, Matsuda A, Matsumoto K, Saito H, Ohya Y. Application of moisturizer to neonates prevents development of atopic dermatitis. *Journal of Allergy and Clinical Immunology* 2014; **134**: 824-30.e6.

15 Lowe AJ, Su JC, Allen KJ, Abramson MJ, Cranswick N, Robertson CF, Forster D, Varigos G, Hamilton S, Kennedy R, Axelrad C, Tang MLK, Dharmage SC. A randomized trial of a barrier lipid replacement strategy for the prevention of atopic dermatitis and allergic sensitization: the PEBBLES pilot study. *British Journal of Dermatology* 2018; **178**: e19-e21.