AMC Bioscad study:

Study population

From January 2015 until August 2016, children with AD aged 0-12 years, were enrolled from the paediatric and dermatology outpatient clinic in the AMC.

Inclusion criteria AD children:

- Clinically diagnosed atopic dermatitis, according to the criteria of Hannifin and Rajka or the UK working party criteria
- Onset of atopic dermatitis between 0-2 years old
- No history of remission of atopic dermatitis without treatment (except emollients) for longer than 1 year

Inclusion criteria healthy controls:

- No inflammatory skin disorder or atopic dermatitis
- No history of atopic dermatitis
- No history of atopic dermatitis in first degree relatives

SCORAD SCORE:

To measure the extent of AD, the rule of nines is applied on a front/back drawing of the patient's inflammatory lesions. The extent can be graded 0-100. The intensity part of the SCORAD index consists of six items: erythema, oedema/ papulation, excoriations, lichenification, oozing/crusts and dryness. Each item can be graded on a scale 0-3. The subjective items include daily pruritus and sleeplessness. Both subjective items can be graded on a 10-cm visual analogue scale. The maximum subjective score is 20. The SCORAD index formula is: A/5 + 7B/2 + C. In this formula A is defined as the extent (0-100), B is defined as the intensity (0-18) and C is defined as the subjective symptoms (0-20). However, since subjective symptoms, such as pruritus and sleep disturbance, have to be estimated by parents, only the ETFAD modified SCORAD score (A/5 +7/2) by excluding the subjective symptoms (objective SCORAD score) was used in this study to eliminate these uncertainties.[28,29]

Preliminary Results:

In total, 53 AD children and 50 healthy controls were found eligible for this study. Population characteristics are shown in Table 2. All children included in the study had a mean age of 4.93 years (median 4, range 0-11, SD 3.34). The male-to-female ratio was 60% for AD children and 47% for healthy controls. The majority of all children had skin type 2. AD severity was classified mild to moderate with a mean objective SCORAD score of 17.6.

Table 2 Patient characteristics

Characteristic	**Healthy controls (*n* = 50)**	**AD Children (*n* = 53)**
Male, n (%)	30 (60.0)	25 (47.2)
Age (mean ± SD years)	5.62 (3.2)	4.28 (3.34)
Skin type		
I, n (%)	3 (6.4)	3 (5.7)
II, n (%)	20 (36.2)	17 (32.1)
III, n (%)	3 (6.4)	5 (9.4)
IV, n (%)	13 (27.7)	10 (18.9)
V, n (%)	2 (4.3)	3 (5.7)
VI, n (%)	9 (19.1)	15 (28.3)
Objective SCORAD score (mean ± SD)		17.63 (9.68)
I		9.97 (1.63)
II		17.56 (4.71)
III		13.04 (5.96)
IV		20.96 (14.87)
V		11.90 (10.31)
VI		19.69 (10.73)
FLG carriers, n (%)	3 (0.06)	13 (24.5)
I, n (%)	--	1 (1.88)
II, n (%)	3	8 (15.09)
III, n (%(	--	2 (3.77)
IV, n (%)	--	1 (1.88)
V, n (%)	--	--
VI, n (%)	--	1 (1.88)

Age and objective SCORAD values are expressed as mean (standard deviation); skin type and male ratio are expressed as frequency (proportion in %). *n* is the number of children in each group.

Natural Moisturising Factors (NMF)

NMF levels were significantly lower in lesional skin compared to healthy skin.

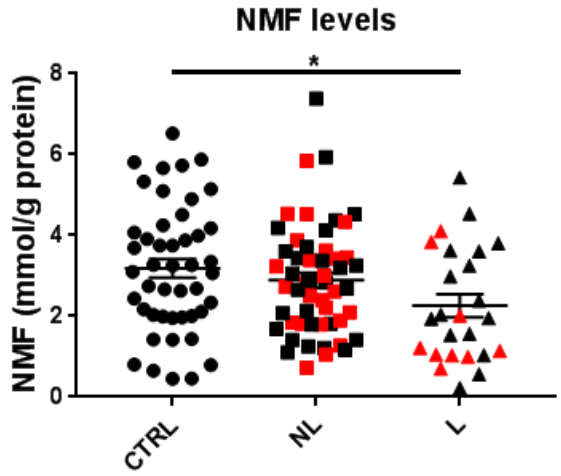

Figure 4 NMF levels in SC of healthy skin of control children (CTRL (●)) and non-lesional (NL ((■)) and lesional (L (▲)) skin of AD children. Red marked chart figures are children with FLG mutation(s).
Line represents mean ± SEM. Significant differences within the groups are depicted with asterisks (*p<0.05).

Between skin types II, IV and VI significant differences between lesional skin and non-lesional skin were found in NMF levels, whereas this difference was not found for healthy skin. An increasing trend in NMF levels is seen in lesional and non-lesional skin for skin types II, IV and VI (Fig. 5).

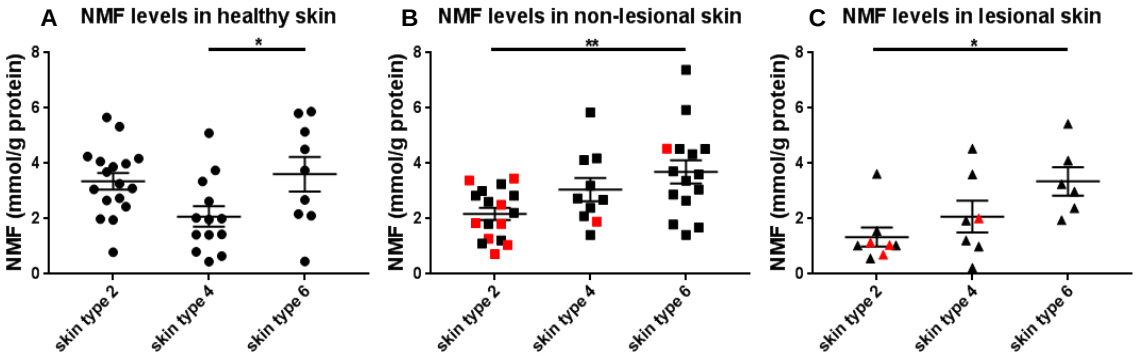

Figure 5 A-C NMF levels in SC of skin type II, IV and VI in healthy skin of control children (●), non-lesional (■) and lesional (▲) skin of AD children. Within each group individual values were stratified by skin type (2, 4 and 6). Marked in red are children with FLG mutation(s). Line represents mean ± SEM. Statistically significant differences between skin types within the groups are indicated with asterisks (*p<0.05; **p<0.01).

Inflammatory mediators

A summary of measured inflammatory mediators is shown in table 2, together with the significance level of the difference between healthy skin (CTRL), non-lesional skin (NL) and lesional skin (L).

Table 2 Concentrations of inflammatory mediators in the SC.

	CTRL (*n* = 47)	NL (*n* = 53)	L (*n* = 24)	CTRL vs NL	CTRL vs L
IL-1α	8.29 (4.49 - 13.32)	5.61 (2.99 - 10.10)	2.245 (1.200 - 4.743)	**0.0445**	**0.0003**
IL-1β	0.0123 (0.0058 - 0.0317)	0.0230 (0.0069 - 0.0330)	0.0045 (0.0246 - 0.0619)	0.7256	**0.01**
IL-2	0.0046 (0 - 0.0086)	0 (0 - 0)	0 (0 - 0)	**<0.0001**	**0.0070**
IL-4	0.0007 (0 - 0.0025)	9.3 e-005 (0 - 0.0007)	0.0004 (0 - 0.0013)	**0.0038**	0.3172
IL-5	0 (0 - 0.0046)	0.0047 (0 - 0.0109)	0.0033 (0.0006 - 0.0102)	0.6926	0.7468
IL-6	0 (0 - 0.0008)	0 (0 - 0.0019)	0.0007 (0 - 0.0051)	0.8284	0.7881
IL-7	0.0045 (0 - 0.0111)	0 (0 - 0)	0 (0 - 0)	**<0.0001**	**<0.0001**
IL-8	0.0285 (0.0175 - 0.0447)	0.0277 (0.0104 - 0.0528)	0.138 (0.0522 - 0.4012)	0.7802	**<0.0001**
IL-8(HA)	0 (0 - 3.507)	0 (0 - 0)	0 (0 - 0)	0.1064	0.1486
IL-10	0.0015 (0 - 0.0033)	0 (0 - 0.0002)	0 (0 - 6.375e-005)	**<0.0001**	**<0.0001**
IL12p40	0.0261 (0.0132 - 0.0463)	0 (0 - 0)	0 (0 - 0)	**<0.0001**	**<0.0001**
IL-12p70	0.0048 (0.0005 - 0.0122)	0 (0 - 0.0020)	0.0015 (0 - 0.0050)	**<0.0001**	**0.0244**
IL-13	0.1018 (0.0221 - 0.1653)	0.0244 (0 - 0.1458)	0.0292 (0 - 0.0989)	0.1354	0.1953
IL-15	0 (0 - 0.0010)	0 (0 - 0.0023)	0 (0 - 0.0004)	0.6593	0.4989
IL-16	0 (0 - 0.1094)	0.0665 (0 - 0.2918)	0.07356 (0 - 0.1729)	**0.0206**	0.5591
IL-17A	0.0041 (0 - 0.0127)	0.0059 (0 - 0.0167)	0.0047 (0 - 0.0194)	0.7088	0.9969
IL-18	0.0172 (0.0056 - 0.0312)	0.0164 (0.0073 - 0.0513)	0.1651 (0.0791 - 5.058)	0.7262	**0.0010**
CXCL10	0.0043 (0 - 0.0130)	0.0083 (0.0048 - 0.0173)	0.0117 (0.0064)	0.4407	0.7703
CCL2	0.0051 (0.0006 - 0.0108)	0.0026 (0 - 0.0098)	0.0110 (0.0041 - 0.0210	0.9313	**0.0083**
CCL3	0 (0 - 0.0785)	0.0489 (0 - 0.1533)	0 (0 - 0.0957)	0.3987	0.8606
CCL4	0.0096 (0 - 0.0689)	0 (0 - 0.0357)	0 (0 - 0.0141)	0.0886	**0.0071**
CCL11	0.0361 (0 - 0.2341)	0.0875 (0 - 0.2231)	0.0578 (0 - 0.1249)	0.346	0.2574
CCL13	0.0828 (0 - 0.167)	0.0719 (0 - 0.1368)	0.0392 (0 - 0.1124)	0.2552	0.1171
CCL17	0.0197 (0.0056 - 0.0328)	0.0126 (0.0003 - 0.0257)	0.0318 (0.0095 - 0.0524)	0.9288	**0.0236**
CCL22	0 (0 - 0.1519)	0 (0 - 0)	0.1919 (0 - 0.5214)	0.9999	**0.0071**
CCL26	0 (0 - 0.0393)	0 (0 - 0)	0 (0 - 0)	0.2514	0.8873
IFN-γ	0.0014 (0 - 0.0248)	0.0044 (0 - 0.0292)	0.0003 (0 - 0.02)	0.8656	0.9402
TNF-α	0 (0 - 0.0028)	0 (0 - 0.0011)	0.0017 (0 - 0.0059)	0.9981	0.1198
GM-CSF	0 (0 - 0.0050)	0.0033 (0 - 0.0072)	0.0014 (0 - 0.0056)	0.206	0.9997

Values are expressed as medians (interquartile ranges). CTRL vs. NL and CTRL vs. NL were tested by one-way ANOVA; NL vs. L was tested by a two-sided Wilcoxon signed rank test. P vales indicate the level of significance. Significant differences are highlighted in bold.