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STRATUM CORNEUM LIPID
COMPOSITION IN ATOPIC
ECZEMA AND ITS ROLE IN THE
SKIN BARRIER FUNCTION

PART III

CHAPTER 5

LAMELLAR LIPID ORGANIZATION AND CERAMIDE COMPOSITION IN STRATUM CORNEUM OF PATIENTS WITH ATOPIC ECZEMA

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The existence of an impaired skin barrier function in atopic eczema (AE, also referred to as atopic dermatitis) has been demonstrated previously, revealing the importance of the skin barrier in the pathophysiology of AE¹. Loss-of-function mutations in the filaggrin gene (*FLG*) are known to be major risk factors for developing AE². However, recent findings report a reduced skin barrier function irrespective of filaggrin genotype, suggesting the importance of additional factors for an impaired skin barrier in AE¹.

The skin barrier is primarily provided by the stratum corneum (SC), consisting of enucleated cells surrounded by lipid regions. SC lipids (primarily ceramides (CERs), cholesterol, and free fatty acids) form two lamellar phases: the short- and long periodicity phase (respectively SPP and LPP) with periodicities of ~6 nm and ~13 nm, respectively. These periodicities were determined by small angle X-ray diffraction (SAXD)³. Barrier properties of the skin may also depend on the lipid organization of the SC lipids and an altered lipid composition or organization may cause a reduced skin barrier in AE. Several studies have reported on the CER composition in AE patients^{4,5}. In the present study, the CER composition and lamellar lipid organization in the SC of AE skin have been simultaneously examined. For AE patients, we observe drastic changes in lipid organization which correlate with changes in CER composition, as compared with control subjects.

The study was conducted in accordance with the Declaration of Helsinki Principles and approved by the Ethical Committee of the Leiden University Medical Center. Subjects gave written informed consent. 6 Caucasian AE patients (23.3 ± 5.2 years; 2 males) and 6 Caucasian subjects without (history of) dermatological disorders (24.7 ± 7.6 years; 1 male)

were included. The subjects did not apply any dermatological products to their forearms for at least one week before the studies. To study the lipid properties irrespective of FLG mutations, we excluded subjects with any of the four most prevalent mutations found in European Caucasians: *2282del4*, *R501X*, *S3247X*, and *R2447X*, analyzed by genotyping⁶.

The lamellar lipid organization was studied using 4 mm biopsies harvested from the ventral forearm of non-lesional skin. The local severity of non-lesional AE skin was evaluated by local SCORing Atopic Dermatitis (SCORAD)⁷, and was 0 for all patients. SC was isolated by trypsin digestion⁸ and analyzed by SAXD using a microfocus beam (European Synchrotron Radiation Facility, Grenoble, France) similarly as described elsewhere⁹. The CER composition was examined in SC harvested by tape stripping non-lesional regions from the ventral forearm (PPS tape, Nichiban, Tokyo, Japan) close to the region of the biopsy. Tape strip numbers 6-9 were extracted, pooled and analyzed by liquid-chromatography (LC) (Alliance 2695, Waters Milford, MA) coupled to mass spectrometry (MS) (TSQ-Quantum, Thermo Finnigan, San Jose, CA)^{10,11}. The nomenclature of used

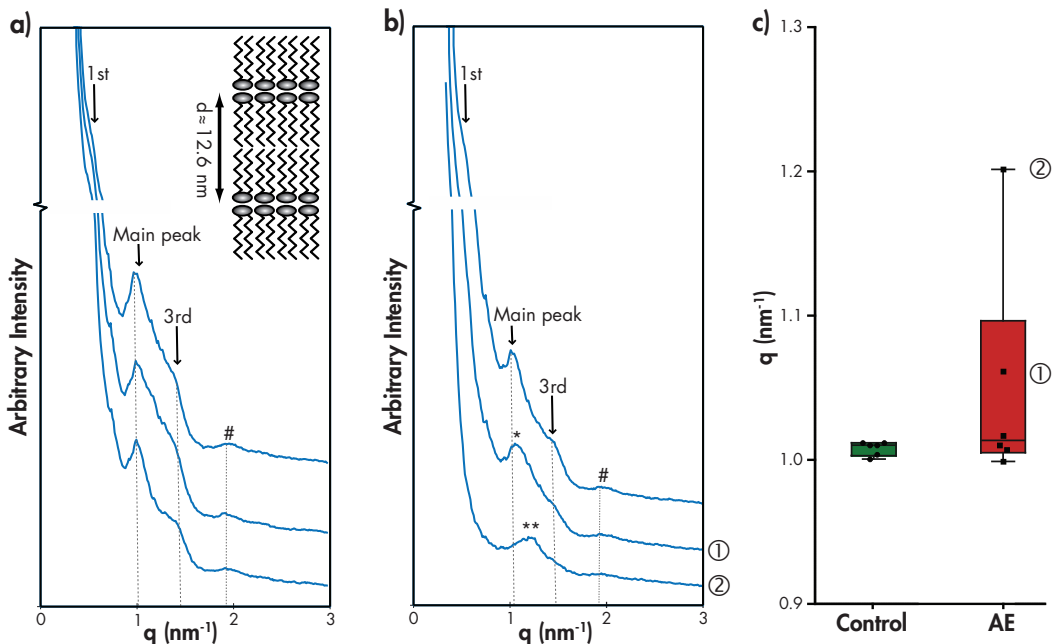


Figure 1: Diffraction patterns of **a)** control subjects, **b)** AE patients and **c)** boxplots showing the variance of the main peak position in both groups. The inset in the upper right corner of **a)** schematically represents the lipid organization and shows the periodicity of the LPP ($d \sim 12.6$ nm). 1 And 2 indicate patients with an altered SAXD profile (indicated by * and **). # Indicates phase separated cholesterol. From the positions of the peaks (q) the repeat distance of the LPP was calculated using the equation $d = n \cdot 2\pi/q_n$ (n = order of diffraction peak). The main peak is also caused by the 1st order diffraction peak of the SPP located at a slightly higher q -value than the 2nd order of the LPP.

CERs is described previously^{1,2}. Briefly, CERs contain a fatty acid chain (either an esterified ω -hydroxy (EO), α -hydroxy (A), or non-hydroxy (N) fatty acid) linked via an amide to a sphingosine chain (either a sphingosine (s), dihydrosphingosine (ds) phytosphingosine (P), or 6-hydroxysphingosine (H)), resulting in 12 CER subclasses.

Figure 1a shows three SC diffraction patterns representative for control subjects. The periodicity of the LPP was obtained from the peak positions³. All control subjects show three LPP peak positions (1st, 2nd and 3rd order) at scattering vector values (q) 0.50, 1.00 (main peak) and 1.45 nm⁻¹ respectively, corresponding to a periodicity of 12.6 nm. The 1st order peak of the SPP also contributes to the main peak, which is located at a slightly higher q -value. Figure 1b shows diffraction patterns of AE patients. The top diffraction pattern closely resembles those of control subjects and is representative for 4 patients. However, two patients (marked 1 and 2) show a different pattern: the central curve shows a shift in the main diffraction peak to a q -value of 1.06 nm⁻¹ (labeled *). The lower curve shows only a single reflection at $q = 1.20$ nm⁻¹ (labeled **). Increased q -values of the peak positions signify shorter periodicities of the lamellar phases in the SC of those patients. The boxplots (Figure 1c) show a small variance between the 6 control subjects, but a large variance within the group of patients.

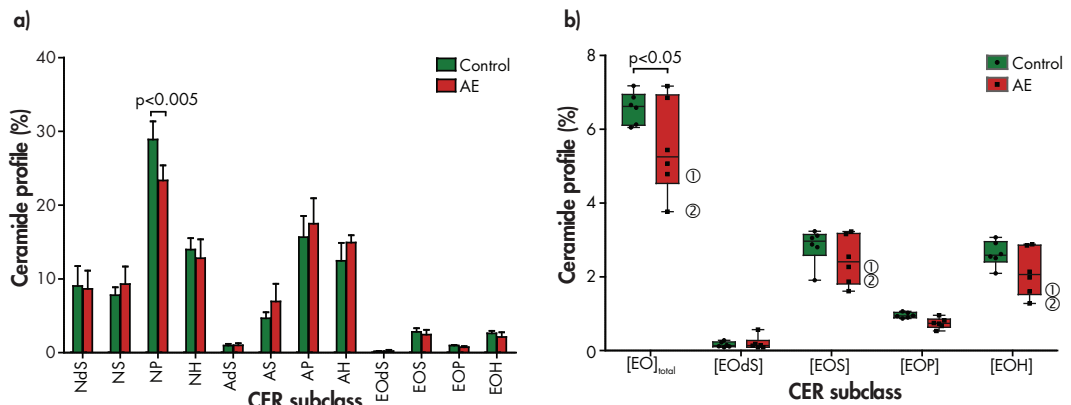


Figure 2: LC/MS analysis on human stratum corneum ceramides obtained from tape strips taken from the ventral forearm. To permit semi-quantitative analysis, two deuterated ceramides (CER [E(18:2)O(30)S(18)]) and CER [N(24)S(18)]) were used as internal standards. **a)** Bar graph of the relative ceramide abundance (in percentages) of every ceramide subclass of control subjects (black bars) and AE patients (grey bars) (mean \pm SD, $n=12$). An unpaired student's t-test showed a significant difference in CER [NP] content between control and AE subjects ($p < 0.005$). **b)** Boxplot of the relative ceramide abundance (in percentages) of the ceramide [EO] subclasses, as well showing the individual data points ($n=12$). White and grey boxes represent respectively control subjects and AE patients. ANOVA showed a significant difference in the total CER [EO] content ($[EOdS] + [EOS] + [EOP] + [EOH]$): $p < 0.05$). Individual AE patients marked as 1 and 2 correspond to the same patients labeled in Figure 1.

Figure 2a shows the CER composition analyzed by LC/MS. All 12 CER subclasses were observed, including the newly identified CER [EODs]¹¹. The observed CER profile is very similar to that observed in previous studies using a fully quantitative method^{4,13}. AE skin showed a significant decrease in the abundance of CER [NP] content ($P < 0.005$, student's t-test) and in the content of long chain CERS (i.e. subclass [EO], Figure 2b, $p < 0.05$, two-way ANOVA), which is in accordance to recent findings⁴. The highest reduction in abundance of CER [EO]-subclasses was observed in patients showing an altered x-ray diffraction pattern: 1 and in particular 2 (Figures 1 and 2). Previous studies have shown that the LPP may be important for the skin barrier function¹⁴: isolated human CERS with a decreased CER [EO] level showed a strong reduction in formation of the LPP¹⁵. This variation in structure results in a more dominant influence of the 1st order SAXD peak of the SPP on the main SAXD peak, which explains the shift in peak position in the diffraction patterns of the two AE patients.

To our knowledge, our studies demonstrate for the first time that a change in CER composition can be associated with a change in the lamellar lipid organization in AE patients compared with control subjects. This association may lead to a better understanding of the altered SC lipid barrier in AE.

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References

- 1 Jakasa I, Koster ES, Calkoen F *et al.* Skin barrier function in healthy subjects and patients with atopic dermatitis in relation to filaggrin loss-of-function mutations. *J Invest Dermatol* 2011; 131: 540-2.
- 2 Palmer CN, Irvine AD, Terron-Kwiatkowski A *et al.* Common loss-of-function variants of the epidermal barrier protein filaggrin are a major predisposing factor for atopic dermatitis. *Nature Genetics* 2006; 38: 441-6.
- 3 Bouwstra JA, Gooris GS, van der Spek JA *et al.* Structural investigations of human stratum corneum by small-angle X-ray scattering. *The Journal of investigative dermatology* 1991; 97: 1005-12.
- 4 Ishikawa J, Narita H, Kondo N *et al.* Changes in the ceramide profile of atopic dermatitis patients. *J Invest Dermatol* 2010; 130: 2511-4.
- 5 Jungersted JM, Scheer H, Mempel M *et al.* Stratum corneum lipids, skin barrier function and filaggrin mutations in patients with atopic eczema. *Allergy* 2010; 65: 911-8.
- 6 Sandilands A, Terron-Kwiatkowski A, Hull PR *et al.* Comprehensive analysis of the gene encoding filaggrin uncovers prevalent and rare mutations in ichthyosis vulgaris and atopic eczema. *Nature Genetics* 2007; 39: 650-4.
- 7 Stalder JF, Taieb A. Severity scoring of atopic dermatitis: the scorad index. Consensus Report of the European Task Force on Atopic Dermatitis. *Dermatology* 1993; 186: 23-31.
- 8 Tanojo H, Bouwstra JA, Junginger HE *et al.* In vitro human skin barrier modulation by fatty acids: Skin permeation and thermal analysis studies. *Pharmaceutical Research* 1997; 14: 42-9.
- 9 Bras W, Dolbnya IP, Detollenaere D *et al.* Recent experiments on a combined small-angle/wide-angle X-ray scattering beam line at the ESRF. *Journal of Applied Crystallography* 2003; 36: 791-4.
- 10 Thakoersing VS, Ponec M, Bouwstra JA. Generation of human skin equivalents under submerged conditions-mimicking the in utero environment. *Tissue Eng Part A* 2010; 16: 1433-41.
- 11 van Smeden J, Hoppel L, van der Heijden R *et al.* LC/MS analysis of stratum corneum lipids: ceramide profiling and discovery. *J Lipid Res* 2011; 52: 1211-21.
- 12 Motta S, Monti M, Sesana S *et al.* Ceramide composition of the psoriatic scale. *Biochim Biophys Acta* 1993; 1182: 147-51.
- 13 Masukawa Y, Narita H, Sato H *et al.* Comprehensive quantification of ceramide species in human stratum corneum. *J Lipid Res* 2009; 50: 1708-19.
- 14 de Jager MW, Gooris GS, Ponec M *et al.* Lipid mixtures prepared with well-defined synthetic ceramides closely mimic the unique stratum corneum lipid phase behavior. *J Lipid Res* 2005; 46: 2649-56.
- 15 Bouwstra JA, Gooris GS, Dubbelaar FE *et al.* Phase behavior of lipid mixtures based on human ceramides: coexistence of crystalline and liquid phases. *Journal of lipid research* 2001; 42: 1759-70.

