

As in Atopic Dermatitis, Nonlesional Skin in Allergic Contact Dermatitis Displays Abnormalities in Barrier Function and Ceramide Content

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TO THE EDITOR

Allergic contact dermatitis (ACD) and atopic dermatitis (AD) are the most common eczematous diseases. Barrier function is impaired, and xerosis is shown in both the inflamed and non-inflamed skin of AD patients (Elias and Steinhoff, 2008). Among stratum corneum (SC) intercellular lipids, which consist of ceramides, cholesterol, and free fatty acid, ceramides (about 50% by weight) play a key role in the skin barrier (Holleran et al., 1991). Accordingly, the SC ceramide content is reduced, even in nonlesional skin of AD patients (Imokawa et al., 1991). Recently, changes in the chain length of *N*-acyl fatty acids in ceramides were recognized as an important factor in explaining quantitative differences in barrier function in AD (van Smeden et al., 2014a, 2014b). If the nonlesional skin of ACD patients also has an impaired SC barrier, a primary inherited defect in the barrier might be emphasized for a pathogenesis of ACD, and moisturizers properly could be helpful in preventing disease exacerbation. Therefore, we evaluated the barrier function and analyzed the SC lipids, including ceramide subtypes, and natural moisturizing factor (NMF) content in the nonlesional skin of ACD or AD.

Subjects with ACD ($n = 25$) and AD ($n = 25$) and healthy control subjects ($n = 35$) were enrolled in this study. The ACD patients showed one or more positive result on patch test, including nickel ($n = 13$), fragrance mix ($n = 9$), rubber ($n = 2$), or cobalt ($n = 1$). To clearly separate AD group from ACD

group, and to avoid the possibility of clinical overlap, the ACD group was limited to patients who did not display typical AD lesions, a personal or family history of atopic diseases, or elevated serum IgE levels. To evaluate the skin

barrier function of the nonlesional skin of ACD or AD patients compared with healthy control subjects, basal transepidermal water loss (TEWL) and SC hydration were measured. There were no differences in the basal TEWL between both disease groups and healthy control subjects (see Supplementary Figure S1a online). SC hydration decreased significantly in AD patients, but not in ACD (see Supplementary Figure S1b). Barrier

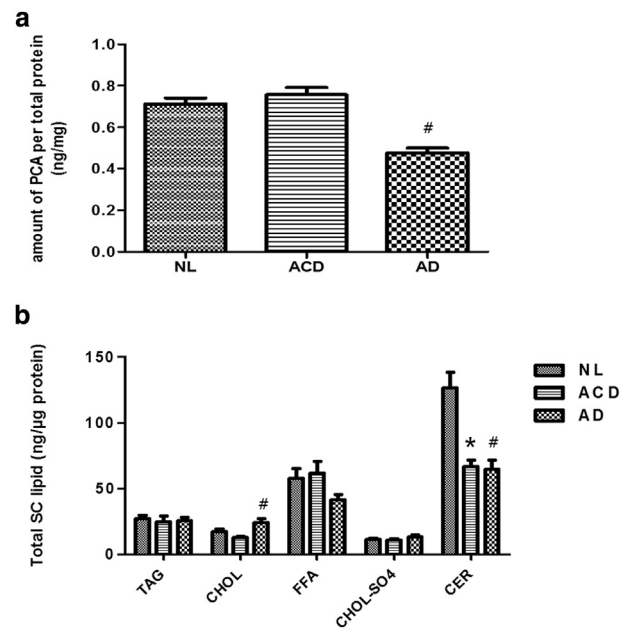


Figure 1. In the corneocytes of the nonlesional skin, PCA significantly decreased only in AD patients, whereas total ceramides significantly decreased in both ACD and AD patients. Quantity of (a) PCA and (b) total SC lipid composition in the corneocytes of the nonlesional skin of ACD or AD patients. (a) Liquid chromatography-tandem mass spectrometry analysis of PCA extracted from the SC of the shin in patients with ACD (male:female = 3:22, age range = 12–68 years, mean age = 35.3 years) or AD (male:female = 12:13, age range = 4–40 years, mean age = 16.9 years) and healthy controls (NL) (male:female = 10:24, age range = 20–54 years, mean age = 33.5 years). The quantity of PCA in AD patients decreased significantly compared with the healthy controls (NL), but not in ACD patients. (b) High-performance thin-layer chromatography analysis for total lipid composition from the SC of the inner forearm in patients with ACD or AD and healthy controls (NL). ACD or AD patients showed significantly decreased CER compared with the healthy controls (NL). * $P < 0.05$, NL versus ACD; # $P < 0.05$, NL versus AD. Results are mean $\pm$ standard error of the mean. ACD, allergic contact dermatitis; AD, atopic dermatitis; CER, ceramides; CHOL, cholesterol; CHOL-SO4, cholesterol sulfate; FFA, free fatty acids; NL, healthy control subjects; PCA, pyrrolidone carboxylic acid; SC, stratum corneum; TAG, triacylglycerol.

Abbreviations: ACD, allergic contact dermatitis; AD, atopic dermatitis; PCA, pyrrolidone carboxylic acid; NMF, natural moisturizing factor; SC, stratum corneum; TEWL, transepidermal water loss

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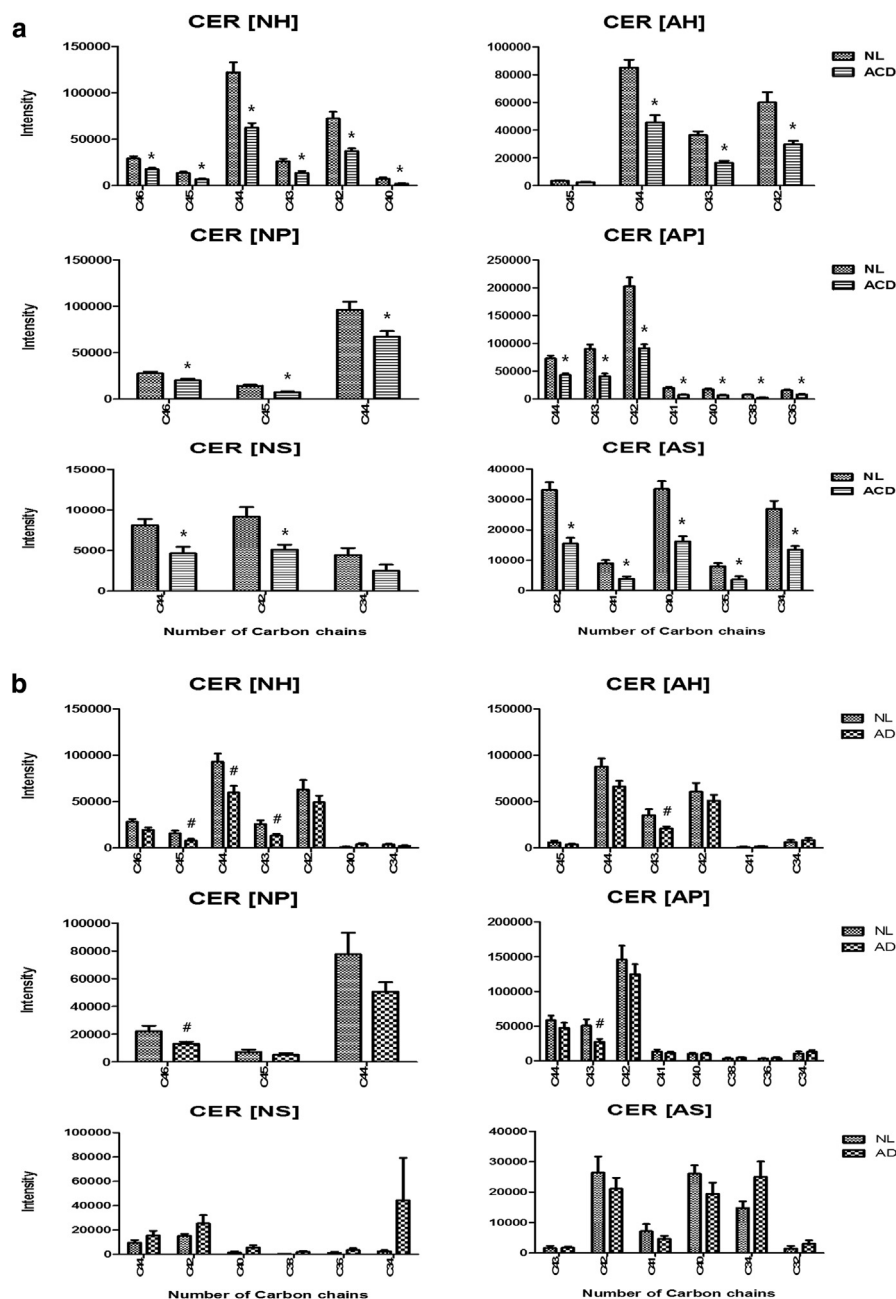


Figure 2. In the SC lipids of nonlesional skin, overall ceramide chain length significantly decreased in ACD patients, whereas long-chain ceramides tended to decrease and short-chain ceramides tended to increase in AD patients. Comparison of each type of ceramide in the SC lipids (a) between the nonlesional skins of ACD patients and the healthy control subjects and (b) between the nonlesional skins of AD patients and healthy control subjects. Ceramide analysis using the liquid chromatography-tandem mass spectrometry system was performed with SC samples. The ceramide subspecies were N-type (CER [NH], CER [NP], CER [NS]) and A-type (CER [AH], CER [AP], CER [AS]). (a) The results of the ACD patients (15 female, range = 21–40 years) were compared with healthy control subjects (20 female, range = 21–40 years) matched by sex and age. In ACD patients, the N/A-type ceramides were significantly decreased in the overall CER chain length. Both the level of long-chain CERs (≥ 42 carbon atoms) and the level of short-chain CERs (< 42 carbon atoms) showed a significant decrease in ACD patients compared with healthy control subjects. (b) The results of the AD patients (12 male, range = 4–40 years) compared with healthy control subjects (5 male, range = 20–28 years) were matched by sex and age. In the AD patients, the levels of long-chain CERs (> 42 carbon atoms) tended to decrease and the level of short-chain CERs (< 42 carbon atoms) tended to increase. * $P < 0.05$, NL vs. ACD; # $P < 0.05$, NL vs. AD. Statistical analysis was performed using unpaired Student *t* test. ACD, allergic contact dermatitis; AD, atopic dermatitis; CER, ceramides; NL, healthy control subjects; SC, stratum corneum.

recovery rates at 3 hours after acute disruption with tape stripping was significantly decreased in both the AD and ACD groups compared with the healthy control subjects (see [Supplementary Figure S1c](#)). Tape stripings with D-Squares (CuDerm, Dallas, TX) were repeated at least 10 times until TEWL reaches over 40 g/m²/h. Although AD subjects as a whole were significantly younger than their healthy control subjects or ACD subjects, their age (range = 4–40 years) did not significantly affect the results of functional barrier studies. Because we compared the ceramide subtypes in the SC lipid after matching for age among the ACD versus AD groups, a bias toward a younger age in the AD subjects would not have occurred. Liquid chromatography-tandem mass spectrometry analysis determined the changes in the pyrrolidone carboxylic acid (PCA) quantity of corneocytes. PCA, though a major component of NMF (12%), is not an indicator of total NMFs, but is representative of NMF ([Scott et al., 1982](#)). It was significantly decreased in AD patients, but not in ACD patients, compared with healthy controls ([Figure 1a](#)), as in previous reports ([Jung et al., 2014; Kezic et al., 2011](#)). In high-performance thin-layer chromatography analyses of SC lipid contents, triacylglycerol and free fatty acids levels did not differ between both disease groups versus the healthy control group. In contrast, there was a significant decrease (about 50%) in the ceramide contents in both disease groups compared with the healthy control group ([Figure 1b](#)). Therefore, the delayed barrier recovery in the nonlesional skin of AD or ACD patients could have resulted from a disorder in ceramide generation.

For ceramide subtypes, we measured the levels of non-hydroxy type (N-type) and α -hydroxy type (A-type) N-acyl fatty acids in ceramides by liquid chromatography-tandem mass spectrometry ([Figure 2](#)). N-type ceramides appeared to be the most abundant species in human SC ([Shin et al., 2014](#)). Among N/A-type ceramides, the ceramide contents of C44 and C42 were mostly detected in the healthy control subjects and ACD patients, but not in AD patients. In ACD patients, however, the overall ceramide chain length

of N/A-type ceramide significantly decreased. In particular, there was a significant decrease not only in long-chain *N*-acyl fatty acids of ceramides (≥ 42 carbon atoms) but also in short-chain fatty acids (< 42 carbon atoms) in the ACD patients (Figure 2a). In AD patients, the decreased levels of long-chain ceramides and the increased levels of short-chain ceramides (Figure 2b) were consistent with previous studies (van Smeden et al., 2014a, 2014b). In AD patients, the levels of long-chain ceramides (≥ 42 carbon atoms) decreased and the level of short-chain ceramides (< 42 carbon atoms) increased compared with healthy control subjects.

Elongases that generate very-long-chain fatty acids 1 (ELOVL1) and 4 (ELOVL4) play an important role in the elongation of free fatty acids above C16 in epidermis (Vasireddy et al., 2007). Protein content of both of these elongases declined in AD epidermis, as described previously (Tawada et al., 2014). This elongation process is suppressed by T helper type 2 cytokines such as IL-13 and IL-4. Thus, AD epidermis might be unable to generate very-long-chain ceramides because of a failure in elongation, according to the observed increase in short-chain ceramides. However, ACD patients may have another mechanism for elongation because of the increased activity of T helper type 1-dominant cytokines. The pathogenesis of ACD may stem from an increased penetration of antigens through the skin barrier, resulting from an inherited disorder of ceramide generation (Novak et al., 2008). *FLG* mutation, a major associated abnormality in AD patients of Northern European ancestry, is significantly associated with ACD patients (especially by nickel) (Thyssen et al., 2008). Intrinsic AD patients have a higher prevalence of metal ACD and a high serum nickel concentration (Yamaguchi et al., 2015). Repeated hapten challenges could change ACD to AD in mice (Man et al., 2008). Therefore, it may be possible that ACD evolves into AD as ACD becomes chronic. This hypothesis will be proven

through future studies. From our result, we conclude that ACD patients present an impaired skin barrier function showing delayed barrier recovery due to insufficient generation of bulk ceramides, regardless of chain length. In contrast to AD, however, ACD presents normal skin hydration because of a normal production of NMFs in the SC.

This study was performed with the approval of the Institutional Review Board of Yonsei University Wonju College of Medicine, Wonju, Korea and written informed patient consent.

CONFLICT OF INTEREST

The authors state no conflict of interest.

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SUPPLEMENTARY MATERIAL

Supplementary material is linked to the online version of the paper at www.jidonline.org, and at <http://dx.doi.org/10.1016/j.jid.2016.10.034>.

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