

Changes in the Ceramide Profile of Atopic Dermatitis Patients

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TO THE EDITOR

We wish to report the characteristic differences that have been identified between the ceramide (CER) profiles of atopic dermatitis (AD) patients and those of healthy individuals.

AD is characterized by impaired stratum corneum (SC) functions, which can be indicated by either an increase in transepidermal water loss (TEWL) or a decrease in water-holding function (capacitance). On the other hand, a close relationship between the impaired SC functions and CERs has been reported (Elias, 1983; Melnik *et al.*, 1988; Yamamoto *et al.*, 1991; Motta *et al.*, 1993; Paige *et al.*, 1994; Di Nardo *et al.*, 1998; Matsumoto *et al.*, 1999; Macheleidt *et al.*, 2002; Cho *et al.*, 2004). More recent research has attempted—but failed—to uncover the diagnostic potential of CER profiling for skin pathologies, including AD (Farwanah *et al.*, 2005).

Human SC CERs can be divided into 11 groups according to their fatty acid and sphingoid structures (Masukawa *et al.*, 2008), as shown in Figure 1a: CER[NDS] contains non-OH fatty acids [N] and dihydrosphingosines [DS]; CER[NS] contains [N] and sphingosines [S]; CER[NH] contains [N] and 6-hydroxy sphingosines [H]; CER[NP] contains [N] and phytosphingosines [P]; CER[ADS] contains α -OH fatty acids [A] and [DS]; CER[AS] contains [A] and [S]; CER[AH] contains [A] and [H]; CER[AP] contains [A] and [P]; CER[EOS] contains ester-linked fatty acids, and ω -OH fatty acids [EO] and [S]; CER[EOH] contains [EO] and [H]; and CER[EOP] contains [EO] and [P]. These classes have been further subdivided into species by chain length.

To date, we have identified 350 species in human SC, using normal-phase liquid chromatography coupled with electrospray ionization-mass spectrometry (Masukawa *et al.*, 2008, 2009; it is worth noting that the former was used to separate CERs into classes based on the polarities). Using the same procedure, we obtained an exhaustive CER profile of the skin of AD patients and healthy individuals to find a characteristic difference between their profiles.

The study was conducted according to the Declaration of Helsinki Principles. The protocol was approved by the ethics committees of the Kao Corporation and Dokkyo Medical University. Informed consent to participate in this study was obtained from the patients. Eight subjects with mild levels of AD (four men and four women; age range, 16–37; mean, 28) and seven healthy individuals with no history of skin disorder (four men and three women; age range, 25–37; mean, 31) were enrolled in this study. First, we evaluated TEWL and capacitance, respectively, using a Tewameter TM 210 and a Corneometer CM 825 (Courage + Khazaka Electronic GmbH, Cologne, Germany) in the affected and unaffected sites on the forearm skin of AD patients and on the forearm skin of healthy individuals. After confirming that TEWL and capacitance were significantly higher and lower, respectively, in the affected sites of the AD patients as compared with those of the unaffected sites of the AD patients and healthy individuals (Figure 1b and c), we sampled SC specimens from these skin sites by performing tape stripping 10 times. CERs were in turn extracted from these specimens and

analyzed with an Agilent 1100 Series LC/MSD single-quadrupole system equipped with an electrospray ionization source, ChemStation software, a 1,100-well-plate autosampler (Agilent Technologies, Palo Alto, CA), and an Inertsil SIL 100A-3, 1.5 mm i.d. $\times$ 150 mm column (GL Science, Tokyo, Japan). These procedures are detailed in our previous reports (Masukawa *et al.*, 2008, 2009).

The levels of total CER as well as those of CER[NH], CER[NP], CER[EOS], CER[EOH], and CER[EOP] classes were found to be significantly lower in the affected sites of AD patients as compared with the normal skin sites of healthy individuals (Figure 1d and e). These findings are consistent with previous findings (Imokawa *et al.*, 1991; Yamamoto *et al.*, 1991; Di Nardo *et al.*, 1998; Bleck *et al.*, 1999; Macheleidt *et al.*, 2002). In addition, this study revealed that the expression levels of CER[AS] were significantly higher in the affected sites of AD patients as compared with the normal sites of healthy individuals.

A significant difference was further detected by dividing each class into species. As a result, we found in AD patients that the larger species (>50 total carbons) of the CER[NS], CER[NDS], CER[NH], CER[AS], and CER[AH] tended to be expressed at lower levels, whereas the smaller species (<40 total carbons) of the CER[NS], CER[NDS], and CER[AS] tended to be expressed at higher levels than the equivalent levels in healthy individuals. A typical example of this tendency is CER[NS] (illustrated in Figure 1f), in which the smaller species (<40 total carbons) were expressed at significantly higher levels and the larger species (>50 total carbons) were expressed at significantly lower levels in the

Abbreviations: AD, atopic dermatitis; CER, ceramide; SC, stratum corneum; TEWL, transepidermal water loss

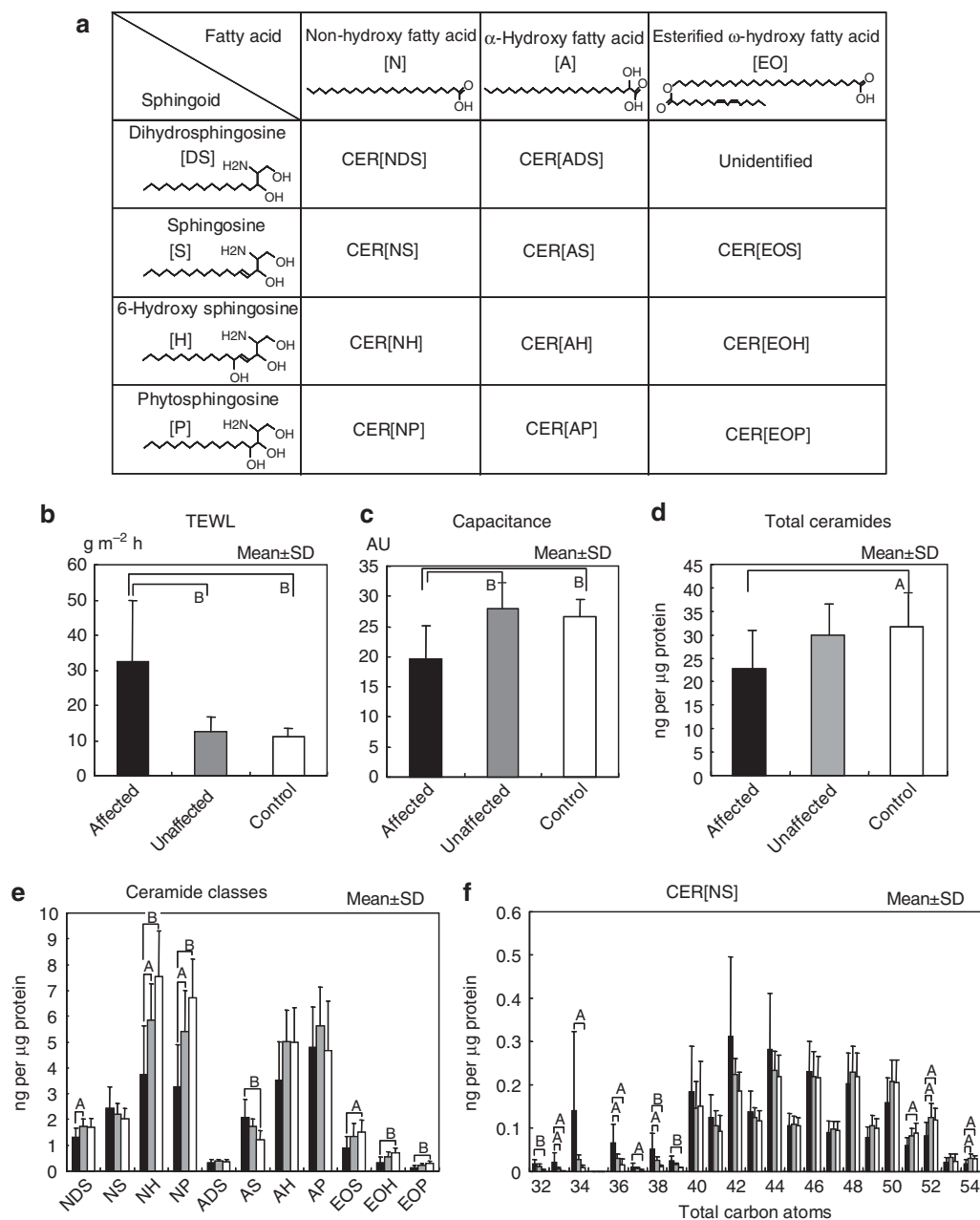


Figure 1. Stratum Corneum (SC) functions and ceramide profiles in AD patients and healthy subjects. (a) Structure and nomenclature of ceramides from the human SC. The mean values of (b) transepidermal water loss (TEWL), (c) capacitance, and the mean levels of (d) total ceramides, of (e) each ceramide class, and of (f) each species in CER[NS] in the affected and unaffected sites of AD patients ($n=8$) and in the normal skin sites of healthy individuals ($n=7$) are indicated by black, gray, and white bars, respectively. The uppercase letters (A, B) denote significant difference by Bonferroni's *post hoc* multiple comparison test ($P<0.05$ and $P<0.01$, respectively).

affected sites of AD patients as compared with those of healthy individuals.

In addition, we detected a parameter that reflects the SC barrier impairments in the CER profile. A correlation with the values of SC barrier functions was detected in the expression level of CER (total, class) and also in the average number of carbon atoms in each CER

class. Table 1a and b summarizes the correlation coefficients and their significance. Previously, a significant negative correlation with TEWL value was found in both the content ratio and the quantity of CER[NP], and a significant positive correlation was found in the content ratio of CER[AH] (Di Nardo *et al.*, 1998). In our study, a significant

correlation with TEWL value was observed but was not limited to the two classes. A strong correlation with the impaired SC barrier functions was found in the expression level of C34-CER[NS] as well as in the average number of carbon atoms of CER[EOP], whereas a strong correlation with the improved SC barrier functions was

Table 1. Correlation coefficient (a) between the SC functional parameters (TEWL and capacitance) and ceramide level, and (b) between the parameters and the average number of carbon atoms per class

	Correlation coefficients	
	TEWL	Capacitance
<i>(a) Levels of CER</i>		
Total CER	−0.675 c	0.629 b
CER[NDS]	−0.638 b	0.615 b
CER[NS]	0.282	−0.232
CER[NH]	−0.730 c	0.613 b
CER[NP]	−0.740 c	0.630 b
CER[ADS]	−0.385	0.417 a
CER[AS]	0.451 a	−0.360
CER[AH]	−0.696 c	0.670 c
CER[AP]	−0.437 a	0.506 a
CER[EOS]	−0.593 b	0.571 b
CER[EOH]	−0.681 c	0.666 c
CER[EOP]	−0.691 c	0.656 c
C34-CER[NS]	0.773 c	−0.563 b
<i>(b) Average numbers of total carbons</i>		
CER[NDS]	−0.862 c	0.659 c
CER[NS]	−0.852 c	0.641 c
CER[NH]	−0.784 c	0.629 b
CER[NP]	−0.190	0.076
CER[ADS]	−0.216	0.159
CER[AS]	−0.637 c	0.512 a
CER[AH]	−0.724 c	0.687 c
CER[AP]	−0.229	0.129
CER[EOS]	0.342	−0.524 a
CER[EOH]	0.045	−0.165
CER[EOP]	0.847 c	−0.633 b

Abbreviations: CER, ceramide; SC, stratum corneum; TEWL, transepidermal water loss. The lowercase letters (a, b, and c) denote significant correlations ($P < 0.05$, $P < 0.01$, and $P < 0.001$, respectively).

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found in the expression levels of CER[NDS], CER[NH], CER[AH], CER[AP], CER[EOS], CER[EOH], and CER[EOP] and in the average number of carbon atoms of CER[NDS], CER[NS], CER[NH], CER[AS], and CER[AH].

Taken together, our findings suggest that the CER profile is a candidate marker for the diagnosis and prognosis of AD.

CONFLICT OF INTEREST

The authors state no conflict of interest.

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