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Original Article



# Alterations of Epidermal Lipid Profiles and Skin Microbiome in Children With Atopic Dermatitis

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## ABSTRACT

**Purpose:** We aimed to investigate epidermal lipid profiles and their association with skin microbiome compositions in children with atopic dermatitis (AD).
**Methods:** Specimens were obtained by skin tape stripping from 27 children with AD and 18 healthy subjects matched for age and sex. Proteins and lipids of stratum corneum samples from nonlesional and lesional skin of AD patients and normal subjects were quantified by liquid chromatography tandem mass spectrometry. Skin microbiome profiles were analyzed using bacterial 16S rRNA sequencing.
**Results:** Ceramides with nonhydroxy fatty acids (FAs) and C18 sphingosine as their sphingoid base (C18-NS-CERs) N-acylated with C16, C18 and C22 FAs, sphingomyelin (SM) N-acylated with C18 FAs, and lysophosphatidylcholine (LPC) with C16 FAs were increased in AD lesional skin compared to those in AD nonlesional skin and that of control subjects (all $P < 0.01$). SMs N-acylated with C16 FAs were increased in AD lesional skin compared to control subjects ($P < 0.05$). The ratio of NS-CERs with long-chain fatty acids (LCFAs) to short-chain fatty acids (SCFAs) (C24-32:C14-22), the ratio of LPC with LCFAs to SCFAs (C24-30:C16-22) as well as the ratio of total esterified omega-hydroxy ceramides to total NS-CERs were negatively correlated with transepidermal water loss (rho coefficients = −0.738, −0.528, and −0.489, respectively; all $P < 0.001$). The proportions of Firmicutes and *Staphylococcus* were positively correlated to SCFAs including NS ceramides (C14-22), SMs (C17-18), and LPCs (C16), while the proportions of Actinobacteria, Proteobacteria, Bacteroidetes, *Corynebacterium*, *Enhydrobacteria*, and *Micrococcus* were negatively correlated to these SCFAs.
**Conclusions:** Our results suggest that pediatric AD skin shows aberrant lipid profiles, and these alterations are associated with skin microbial dysbiosis and cutaneous barrier dysfunction.

**Keywords:** Atopic dermatitis; child; microbiota; *Staphylococcus*; ceramides; lipids; fatty acids

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**Disclosure**

Dr. Leung has consulted for Regeneron, Sanofi, Novartis and Genzyme. Dr. Berdyshev has research contracts from LEO Pharmaceutical and Sanofi.

## INTRODUCTION

The stratum corneum (SC) is the outermost part of the skin and acts mainly as a barrier by preventing the entry of microbes, allergens, and irritants into the human body.[1,2] The structure of the SC consists of corneocytes and intercellular lipid matrix that form a "brick and mortar" arrangement.[1] The lipid matrix consists of ceramides (CERs), free fatty acids (FFAs), cholesterol, and triglycerides released from the lamellar bodies of keratinocytes.[3-5] Diverse microbial communities colonize in the human skin and interact with keratinocytes and immune cells to maintain skin homeostasis.[2,6] Skin barrier dysfunction caused by defects in epidermal structure is crucial in the pathogenesis of atopic dermatitis (AD) together with immune dysregulation and skin microbial dysbiosis.[7]

AD affects up to 20% of children, and approximately 60% of AD cases begin during the first two years of life.[8,9] It has been reported that the pathophysiology of AD in children is different from that of AD in adults.[10,11] Although both pediatric and adult AD skin shows Th2 immunity, adult AD shows more prominent epidermal differentiation complex defects and lesional predilection for the hands and face.[10,11] Recently, new insights into the pathophysiology of AD have emphasized the crucial roles of abnormalities in the epidermal lipid layer as well as skin microbial dysbiosis in adults.[12] AD skin showed dysbiosis such as an increased proportion of *Staphylococcus* and reduced proportions of *Streptococcus, Acinetobacter, Corynebacterium*, and *Propionibacterium* during AD flares, resulting in a loss of bacterial diversity.[12-14] AD skin also displayed an altered epidermal lipid composition such as accumulation of short-chain CERs and reduction in very-long-chain CERs, leading to an aberrant lipid organization in the extracellular layers and increased transepidermal water loss (TEWL).[15,16] A recent study demonstrated that the levels of long-chain CERs were lower in the skin of AD patients colonized with *Staphylococcus aureus* than in those without, suggesting the association of changes in lipid composition with *S. aureus* colonization and barrier dysfunction.[17]

Previously published papers focused on profiles of lipids and microbiomes in adult AD patients, and demonstrated significant differences in lipid profiles and microbiome composition between young children and adults.[17-20] Additionally, children with AD showed higher levels of interleukin (IL)-17A, IL-19, and LL37 in lesional and nonlesional skin than adults with AD, suggesting that the skin phenotype of pediatric AD differs immunologically from that of adult AD.[21] Moreover, the abundance of specific bacteria such as *Propionibacteria* and *Corynebacteria* was correlated with epidermal lipid composition in German patients with AD.[18] Therefore, we hypothesized that epidermal lipid profiles and skin microbiomes may vary between children and adults as well as among races. In the present study, we aimed to investigate epidermal lipid profiles and their association with skin microbiome compositions in Korean children with AD.

## MATERIALS AND METHODS

### Study subjects

Skin samples were obtained using tape stripping from 27 children with AD and 18 healthy subjects matched for age and sex. The diagnosis of AD was based on the criteria defined by Hanifin and Rajka.[22] Parents were asked to respond to questionnaires to obtain demographic data, including a personal history of food allergy, allergic rhinitis, or asthma. The healthy control group was comprised of subjects with no personal or family history of atopy and

skin diseases. AD patients were assessed by allergists using the SCORing Atopic Dermatitis (SCORAD), ranging from 0 to 103.[23] Patients had not received topical corticosteroids, topical calcineurin inhibitors, topical or oral antibiotics for at least 1 week prior to enrollment.[24] Patients were not treated with systemic immunosuppressive medications for more than 1 month prior to enrollment. TEWL was measured on the volar surface of the right forearm by a Tewameter TM300 (Courage & Khazaka, Köln, Germany) in a test room with relative humidity of 40%–50% and temperature of 20°C–22°C. This study was approved by the Institutional Review Boards (IRBs) at Samsung Medical Center, Pusan National University Hospital, and Inje University-Sanggye Paik Hospital (IRB No. SMC-2018-03-041, H-1808-021-070, and SGPAIK 2018-06-005-001, respectively), and written informed consent was obtained from all parents and/or patients prior to participation in this study.

## Laboratory tests

Blood samples were collected at the initial visit. The specific IgE antibodies to *Dermatophagoides pteronyssinus*, *D. farinae*, egg white, cow milk, wheat, soy, and peanut were measured with the ImmunoCAP system (ThermoFisher Scientific Inc., Waltham, MA, USA), and concentrations ≥ 0.35 kU/L determined sensitization.

## Collection of skin tape stripping (STS) samples

A total of 4 consecutive D-Squame® tape discs (22-mm diameter, CuDerm, Dallas, TX, USA) were applied to the volar surface of the forearm as described previously.[25] STS samples were collected from lesional and nonlesional areas of the AD patients as well as from the skin of healthy control subjects. Upon application of the first tape disc, 4 marks were placed around the disc with a pen, so that subsequent discs could be applied to the same location. Each tape disc was placed in a separate well of 6-well plates allocated for the sample collection. Tape strips 3 and 4 were designated for lipid analysis and stored at −80°C until the lipids were extracted.

## Tape strip processing for lipid extraction and protein estimation

Samples from tape strips 3 and 4 were removed by scraping the tape strips in 2 mL of a water-methanol (9:1, v/v) mixture in a petri dish with a rubber cell scraper. Tape strip processing was performed according to the previously published protocol.[19] Floating SC particles were transferred into 8-mL glass screw-cap tubes and then subjected to a modified Bligh and Dyer extraction.[19,26] A mixture of lipid internal standards was added during the initial step of lipid extraction to ensure absolute quantitation of the targeted lipid subclasses. In brief, extraction was performed overnight by adding 0.5 mL methanol and 1 mL chloroform; then, phase separation was achieved by vortexing and centrifugation after adding 1.5 mL chloroform and 1.35 mL 2% formic acid. The upper water-methanol phase was carefully removed, and the bottom chloroform phase was collected with minimal disturbance to the protein interphase and evaporated by a stream of nitrogen. Lipids were dissolved in 0.2 mL methanol for liquid chromatography electrospray ionization tandem mass spectrometry (LC-ESI-MS/MS) analyses. The residual protein denaturates/pellets were dried by a stream of nitrogen and hydrolyzed with 0.5 mL of 1N sodium hydroxide for 3 hours at 55°C.[26,27]

Tubes were periodically vortexed. After hydrolysis, the sodium hydroxide was neutralized with 0.5 mL of 21N hydrochloric acid, the hydrolyzed proteins were centrifuged at 2,000 g for 5 minutes, and protein concentrations were determined using a detergent compatible (DC) protein assay kit (Bio-Rad; Hercules, CA, USA) with bovine serum albumin (BSA) as a protein standard. It was determined that hydrolysis of BSA is not needed to determine concentrations by DC protein assay, as both nonhydrolyzed and hydrolyzed BSA provide the same readings.


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### Lipid analyses

CERs, lysophosphatidylcholines (LPCs), and sphingomyelins (SMs) from human skin were identified and quantified using a targeted LC-ESI-MS/MS approach on a Sciex 6500 QTRAP mass spectrometer coupled with a Shimadzu Nexera X2 UHPLC system, as described previously.[19]

### Genomic DNA extraction and bacterial 16S rRNA sequencing

Skin swab samples were obtained from the nonlesional and lesional volar forearm skin of AD patients and the normal skin of healthy control subjects. The whole bacterial genomic DNA was extracted using a Maxwell® RSC PureFood GMO and Authentication Kit (Promega, Madison, WI, USA) according to the manufacture's protocol. The hypervariable regions V3-V4 of the bacterial 16S rRNA gene were amplified from skin swab samples and sequenced on the Illumina MiSeq platform (Illumina, San Diego, CA, USA). The 16S rRNA gene sequence data were analyzed using the Quantitative Insights Into Microbial Ecology (QIIME) software package (v1.9.1).[28] Using qualified sequences (Phred ≥ Q20), the operational taxonomic units were identified and quantified using the open reference method that maps sequences with 97% identity to known sequences in the Greengenes database (v13_8) using UCLUST alignment algorithms and the EzBioCloud database (http://www.ezbiocloud.net).[29-31]

### Statistical analyses

Data were analyzed using SPSS for Windows (version 27.0; SPSS, Armonk, NY, USA). Categorical data are presented as numbers and percentages. Continuous data are presented as arithmetic means ± standard deviations (SD). Categorical variables between groups were compared using the chi-square test or Fisher's exact test. Data were analyzed by ANOVA with post-hoc tests to compare the nonlesional and lesional skin of children with AD with the skin of controls. Statistical differences between groups were determined using an unpaired t-test or Mann-Whitney *U* test, depending on the distribution of the data. The correlation between the two variables was calculated using Spearman's correlation. Bonferroni's correction was applied to account for multiple comparisons. A $P < 0.05$ was considered significant.

## RESULTS

### Aberrant SC lipid profiles in the skin of children with AD

We collected skin samples from 27 children with AD (Mean± SD) (8.3 ± 4.2 yr) and 18 healthy children (6.8 ± 4.1 yr) (**Table**). The mean (±SD) SCORAD score of the AD patients was 28.9 ± 15.1. There were no significant differences in sex and family history of allergic diseases between the AD and control groups (**Table**). The amounts of total CERs and esterified omega-hydroxy ceramides (EOS-CERs) were lower in the AD lesional skin than in AD nonlesional and healthy control skin (**Fig. 1A**, all $P<0.01$). In contrast, the amount of SMs was higher in the AD lesional than in AD nonlesional and healthy skin (**Fig. 1A**, $P<0.01$). However, no difference was observed in the amounts of CERs with nonhydroxy fatty acids (FAs) with C18 sphingosine as their sphingoid base (C18-NS-CERs) and LPCs between nonlesional and lesional skin in AD patients (**Fig. 1A**).

Lipid molecular species with short-chain fatty acids (SCFAs, C16-C18) of C18-NS-CERs, SMs, and LPCs were increased in AD skin, whereas lipid species with long-chain fatty acids (LCFAs) were globally decreased (**Fig. 1B-D**). C18-NS-CER N-acylated with C16, C18 and C22 FAs (**Fig. 1B**, $P<0.01$), SM N-acylated with C18 FA (**Fig. 1C**, $P<0.01$), and LPCs with C16 FAs (**Fig. 1D**, $P<0.01$) were increased in the AD lesional skin compared to those in nonlesional


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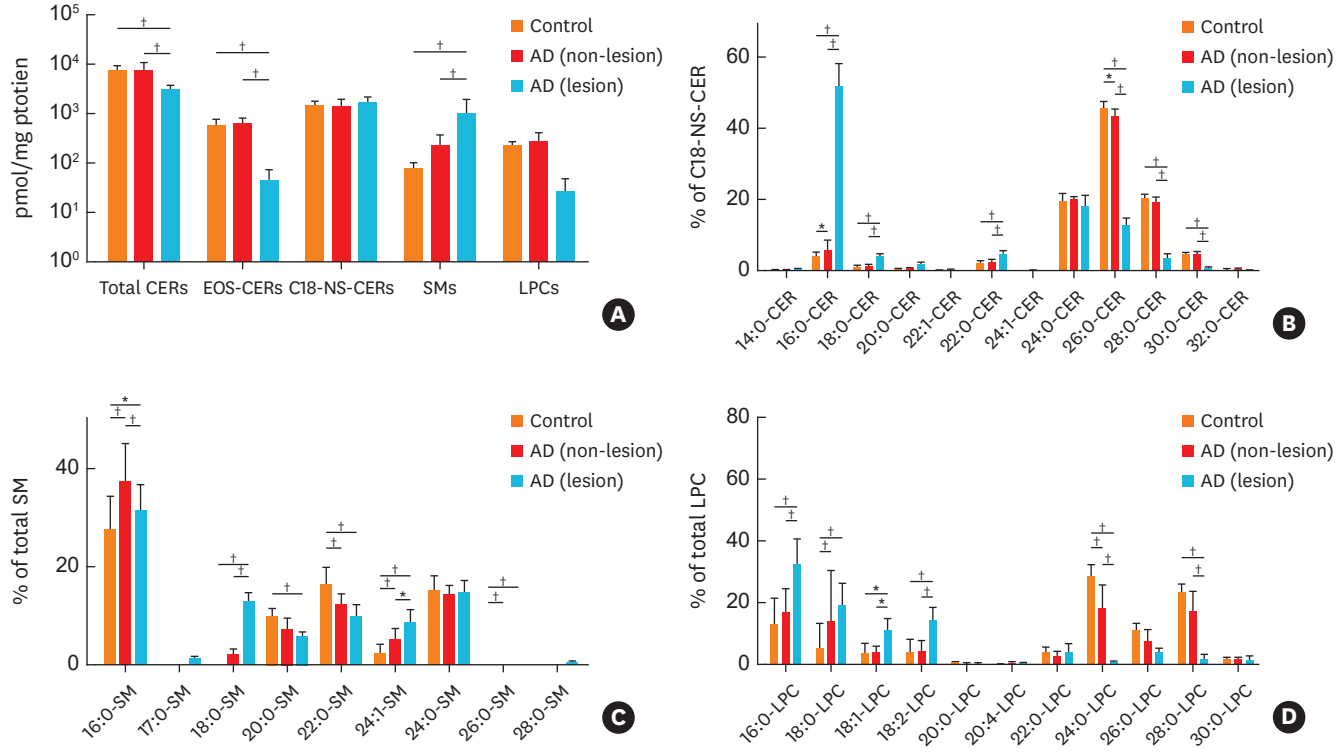


**Fig. 1.** The lipid composition (A) and proportion of ceramides with C18 sphingosine as their sphingoid base and nonhydroxy fatty acids (B), sphingomyelins (C), and lysophosphatidylcholine (D) in the skin of normal subjects and children with atopic dermatitis. The data are shown as the median and interquartile range.
AD, atopic dermatitis; CER, ceramide; EOS-CER, ester-linked omega-hydroxy ceramide, C18-NS-CER, ceramide with C18 sphingosine as its sphingoid base and nonhydroxy fatty acids; SM, sphingomyelin; LPC, lysophosphatidylcholine.
*$P$ < 0.05 and †$P$ < 0.01 by one-way ANOVA with the Tukey-Kramer test. Bonferroni's correction was applied to account for multiple comparisons.

**Table.** The baseline clinical characteristics of subjects (N = 45)

| Variables | Control (n = 18) | AD (n = 27) | *P* value |
|---|---|---|---|
| Age (yr) | 6.8 ± 4.1* | 8.3 ± 4.2* | 0.208 |
| Male (%) | 9 (50.0) | 16 (59.3) | 0.540 |
| Comorbid conditions | | | |
| Food allergy | 0 (0) | 10 (37.0) | 0.003 |
| Allergic rhinitis | 0 (0) | 9 (33.3) | 0.007 |
| Asthma | 0 (0) | 4 (14.8) | 0.138 |
| Allergic sensitization (%) | 0 (0) | 16 (59.3) | < 0.001 |
| Family history of allergic diseases (%) | 12 (66.7) | 20 (74.1) | 0.591 |
| SCORAD | NA | 28.9 ± 15.1 | |
| TEWL (g/m²·h) | | | |
| Nonlesional skin | 16.6 ± 4.0 | 31.8 ± 11.3 | < 0.001 |
| Lesional skin | NA | 51.1 ± 13.8 | |
| pH | | | |
| Nonlesional skin | 4.5 ± 0.3 | 4.6 ± 0.2 | 0.138 |
| Lesional skin | NA | 4.8 ± 0.4 | |
| Corneometer (A.U.) | | | |
| Nonlesional | 37.8 ± 10.1 | 32.0 ± 10.1 | 0.077 |
| Lesional | NA | 11.9 ± 9.8 | |

AD, atopic dermatitis; SCORAD, scoring atopic dermatitis; TEWL, transepidermal water loss; NA, not available.
*Data are presented as mean ± standard deviation.

skin of AD patients and control subjects. SM N-acylated with C16 FA was greater in AD lesional skin than in control subjects ($P$<0.05). Additionally, C18-NS-CERs with C16 FA ($P$<0.05), SM with C16 FA ($P$<0.01), and LPCs with C18 FAs ($P$<0.01) were increased in the AD nonlesional skin compared to the skin of control subjects. On the other hand, LCFAs


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including C18-NS-CERs (with C26-30 FAs, **Fig. 1B**, $P<0.01$) and LPCs (with C24 and C28 FAs, **Fig. 1C**, $P<0.01$) were decreased in the nonlesional and lesional skin of AD patients compared to the skin of control subjects. The AD lesional skin also showed a lower proportion of SMs with C20-22 and C26 FAs than the skin of healthy children ($P<0.01$). Additionally, C18-NS-CER with C26 FA ($P<0.05$), SMs with C22 and C26 FAs ($P<0.01$), and LPCs with C24 FAs ($P<0.01$) were decreased in the AD nonlesional skin compared to the skin of control subjects. However, SM with C24:1 FA was increased in the AD lesional and nonlesional skin compared to the skin of control subjects (**Fig. 1C**, $P<0.05$).

## An altered lipid profile is associated with increased TEWL in AD skin

The correlations between TEWL and SC lipids such as C18-NS-CERs, EOS-CERs, SMs, and LPCs were analyzed in children. As a result, the ratios of NS-CER with LCFAs to SCFAs (C24-32:C14-22) and LPC with LCFAs to SCFAs (C24-C30:C16-C22), as well as the ratio of total EOS-CER to total NS-CER were negatively correlated with TEWL (rho coefficients= -0.738, -0.528, and -0.489, respectively; all $P<0.001$) (**Fig. 2**). However, there was no correlation between the ratio of SMs with LCFAs to SCFAs (C24-C28:C16-C22) and TEWL (rho coefficients= -0.147 and $P=0.228$).

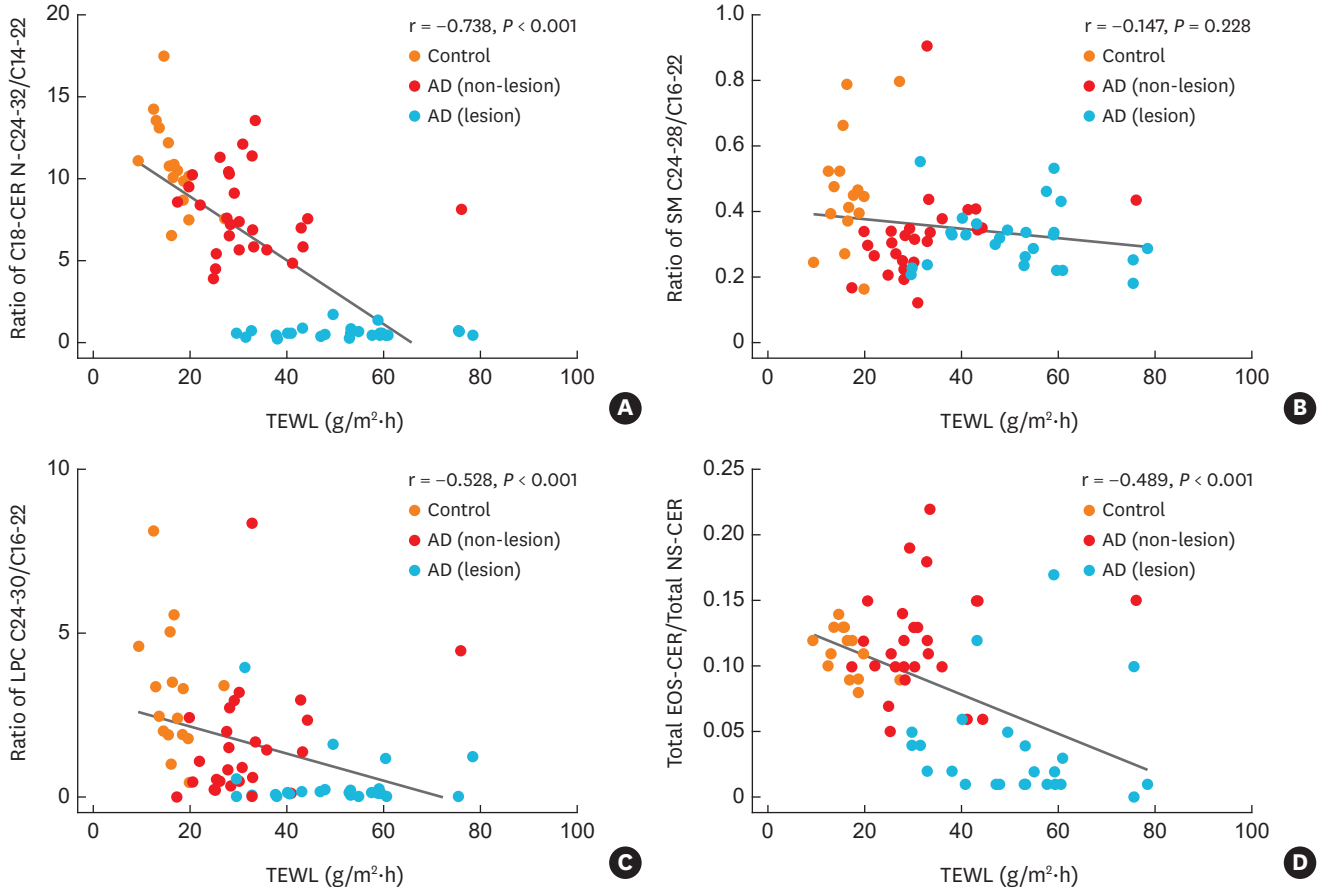


**Fig. 2.** Association of transepidermal water loss with ratios of long-chain to short-chain fatty acid-containing ceramides with C18 sphingosine as their sphingoid base and nonhydroxy fatty acids (A), sphingomyelins (B), lysophosphatidylcholine (C), and the proportion of total ester-linked omega-hydroxy ceramides to total non-hydroxy fatty acid-containing ceramides (D) in the skin of atopic dermatitis patients and healthy subjects.
AD, atopic dermatitis; TEWL, transepidermal water loss; CER, ceramide; EOS-CER, ester-linked omega-hydroxy ceramide, C18-NS-CER, ceramide with C18 sphingosine as its sphingoid base and nonhydroxy fatty acids; SM, sphingomyelin; LPC, lysophosphatidylcholine.


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## Microbiome profiling in the skin from AD patients and healthy controls

In the skin of AD patients and healthy controls, 6 prevalent bacterial phyla, Firmicutes, Actinobacteria, Proteobacteria, Bacteroidetes, Cyanobacteria, and Fusobacteria, made up more than 98% of each subject's microbiota (**Fig. 3A**). The lesional skin of AD patients displayed a significantly increased abundance of Firmicutes and decreased abundance of Actinobacteria and Proteobacteria at the phylum level compared to the nonlesional skin of AD patients and control subjects (**Fig. 3A**, all $P<0.01$). The AD lesional skin also showed a lower Bacteroidetes abundance than the skin of control subjects (**Fig. 3A**, $P<0.05$). At the genus level, a higher *Staphylococcus* abundance ($P<0.01$) and a lower *Corynebacterium* abundance ($P<0.05$) were found in the AD lesional skin compared to the nonlesional skin of AD patients and to that of control subjects (**Fig. 3B**). Additionally, the lesional skin of AD patients showed a decreased abundance of *Micrococcus* compared to the skin of control subjects (**Fig. 3B**, $P<0.05$). However, no differences were found in the relative abundance of bacterial phyla and genera between AD nonlesional skin and that of the control subjects.

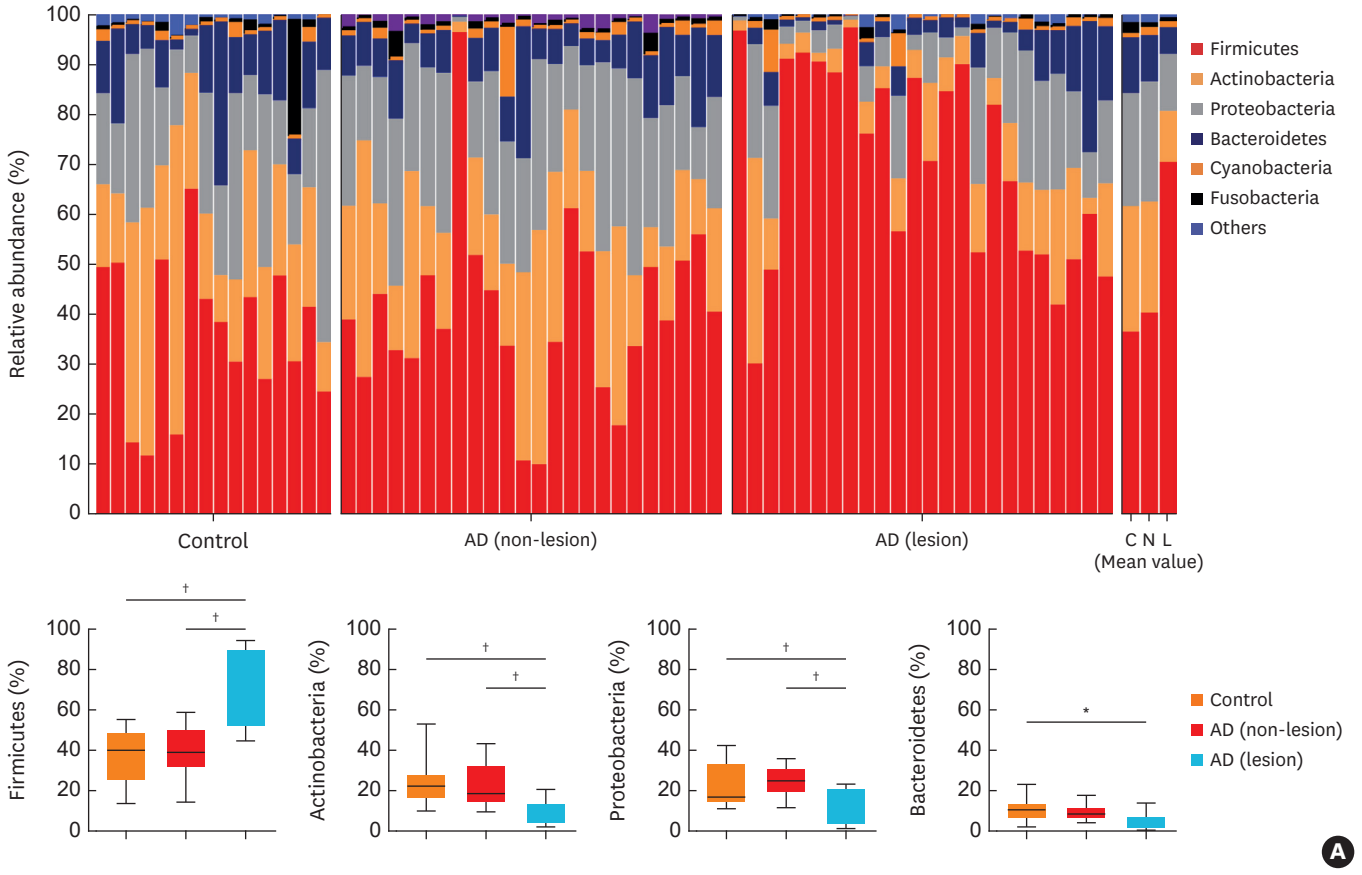


**Fig. 3.** Skin microbiome composition at the phylum (A) and genus (B) levels in nonlesional and lesional skin of atopic dermatitis patients and the skin of healthy subjects.
AD, atopic dermatitis; C, control subjects; N, non-lesional skin of atopic dermatitis patients; L, lesional skin of atopic dermatitis patients. Lines within boxes display median values, and bars represent 10th and 90th percentiles.
*$P < 0.05$ and †$P < 0.01$ by one-way ANOVA with the Tukey-Kramer test.

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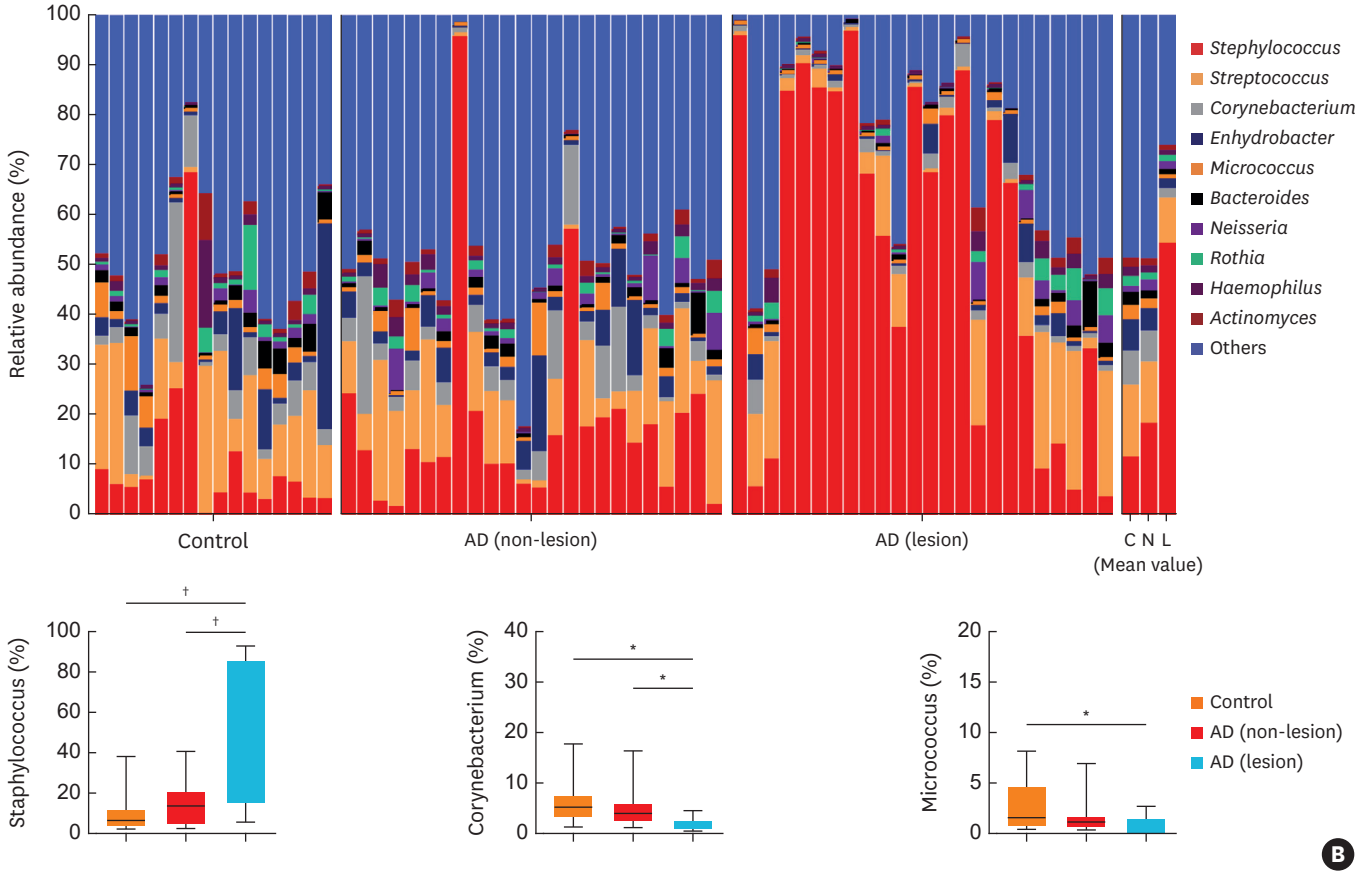


**Fig. 3.** (Continued) Skin microbiome composition at the phylum (A) and genus (B) levels in nonlesional and lesional skin of atopic dermatitis patients and the skin of healthy subjects.
AD, atopic dermatitis; C, control subjects; N, non-lesional skin of atopic dermatitis patients; L, lesional skin of atopic dermatitis patients. Lines within boxes display median values, and bars represent 10th and 90th percentiles.
*$P$ < 0.05 and †$P$ < 0.01 by one-way ANOVA with the Tukey-Kramer test.

## Skin dysbiosis is associated with aberrant skin lipid compositions in pediatric AD skin

At the phylum level, the proportion of Firmicutes was positively correlated with C18-NS-CERs containing saturated SCFAs (C14-C22), with SMs with SCFAs (C17-C18), and with LPCs containing SCFAs (C16), while the proportions of Actinobacteria, Proteobacteria, and Bacteroidetes were negatively correlated with these groups of lipids (**Fig. 4**). In contrast, the relative abundance of Firmicutes was negatively associated with NS-CERs containing saturated LCFAs (C26-C32), with SMs containing C20, C22 and C26 FAs, and with LPCs containing C20, C24 and C28 FAs, while the relative abundance of Actinobacteria, Proteobacteria, and Bacteroidetes was positively associated with all those lipids that contain long-chain FAs. The relative abundance of Fusobacteria showed a positive correlation with C18-NS-CERs containing LCFAs (C26 and C32) and SMs containing C22 FAs, and a negative correlation with C18-NS-CERs containing SCFAs (C14-C22), and with SMs containing C17 FAs. Similarly, at the genus level, the proportion of *Staphylococcus* was positively correlated with C18-NS-CERs containing saturated SCFAs (C14-C22), SMs with C17-C18 FAs, while the proportions of *Corynebacterium, Enhydrobacteria*, *Micrococcus,* and *Bacteroides* were negatively correlated with these group of lipids containing SCFAs. The relative abundance of *Staphylococcus* showed a negative correlation with C18-NS-CERs containing C26-C32 FAs,


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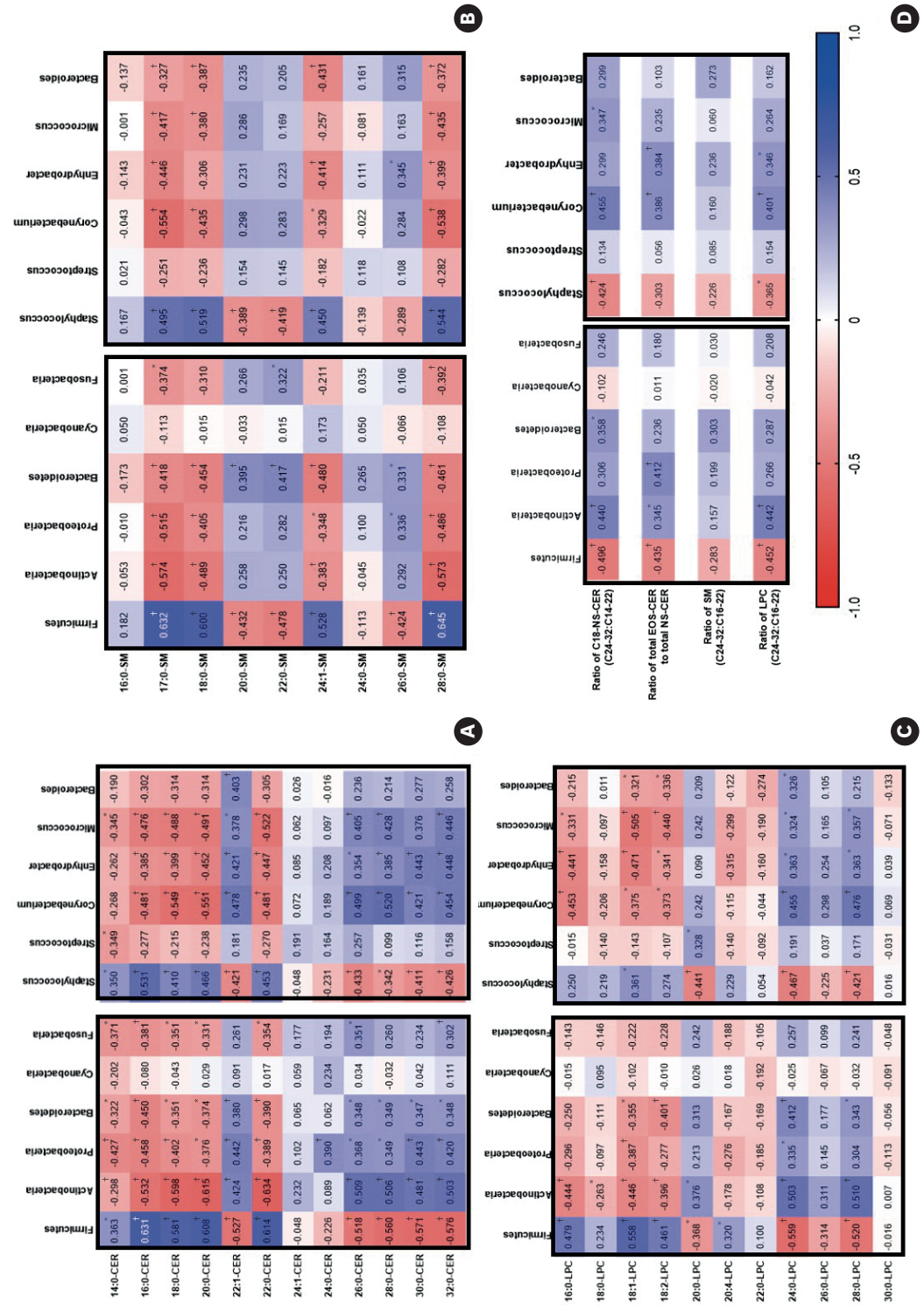


**Fig. 4.** A heat map shows the correlation between microbiome (phylum and genus) and lipid profiles such as the proportions of ceramides with C18 sphingosine as their sphingoid base and nonhydroxy fatty acids (NS ceramides) (A), sphingomyelins (B), lysophosphatidylcholine (C), and ratios of long-chain to short-chain fatty acids (D) in the skin of atopic dermatitis patients and healthy subjects. Spearman correlation coefficients between lipid profiles and skin microbiome are included.
CER, ceramide; EOS-CER, ester-linked omega-hydroxy ceramide, C18-NS-CER, ceramide with C18 sphingosine as its sphingoid base and nonhydroxy fatty acids; SM, sphingomyelin; LPC, lysophosphatidylcholine.
*$P < 0.05$; †$P < 0.01$. Bonferroni's correction was applied to account for multiple comparisons.



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SMs containing C20-C22 FAs, and LPCs containing C20, C24, and C28 FAs. Additionally, the relative abundance of *Streptococcus* showed a positive correlation with LPCs containing C20 FAs, and a negative correlation with C18-NS-CERs containing C14 FAs. There was also a negative correlation between LPCs with C16 FAs and the proportions of *Corynebacterium, Enhydrobacteria*, and *Micrococcus* (**Fig. 4**).

C18-NS-CER with monounsaturated SCFAs (C22:1) showed a negative correlation with the abundance of Firmicutes and *Staphylococcus* and a positive correlation with the abundance of Actinobacteria, Proteobacteria, Bacteroidetes, *Corynebacterium, Enhydrobacteria*, *Micrococcus,* and *Bacteroides.* SM with monounsaturated FAs (C24:1) had a positive relationship with the abundance of Firmicutes and *Staphylococcus* and a negative relationship with the abundance of the phyla Actinobacteria, Proteobacteria, and Bacteroidetes, and the genera *Corynebacterium, Enhydrobacter*, and *Bacteroides.* LPCs with monounsaturated SCFA (C18:1) and polyunsaturated arachidonic acid (C20:4) showed a positive correlation with Firmicutes, while LPCs with monounsaturated FAs (C18:1) revealed a negative correlation with the abundance of the phyla Actinobacteria, Proteobacteria, and Bacteroidetes.

SM with very-long-chain C28:0 FA displayed a positive association with the proportions of Firmicutes and *Staphylococcus* and a negative association with the proportions of Actinobacteria, Proteobacteria, and Bacteroidetes, and the genera *Corynebacterium*, *Enhydrobacter*, *Micrococcus*, and *Bacteroides*. We also found that Firmicutes at the phylum level was negatively correlated with the ratio of C18-NS-CER with LCFAs (C24-C32) to C18-NS-CER with SCFAs (C14-C22), with the ratio of total EOS-CERs to total NS-CERs, and with the ratio of long-chain to short-chain LPCs (C24-C32:C16-C22 FAs). Similarly, the abundance of *Staphylococcus* at the genus level was negatively correlated with the ratio of C18-NS-CER with LCFAs (C24-C32) to C18-NS-CER with SCFAs (C14-C22), and with the ratio of long-chain to short-chain LPCs (C24-C32:C16-C22 FAs).

## DISCUSSION

SC lipids play an important role as a part of the epidermal barrier because the lipid envelope acts as a hydrophobic impermeable layer of matured corneocytes to prevent loss of water and natural moisturizing factors.[32] However, few studies have been conducted to investigate epidermal lipid compositions in AD children. In the present study, nonlesional and lesional SCs of pediatric AD patients displayed increased proportions of NS CERs, LPCs, and SMs with SCFAs as well as a simultaneous reduction in the proportion of corresponding long-chain species compared to the SCs of normal children. These changes in SCFAs and LCFAs were more prominent in the lesional skin than in nonlesional areas of AD patients. Furthermore, an increased proportion of SC lipids with SCFAs was associated with epidermal barrier dysfunction and skin microbial dysbiosis. In addition to the observations of Baurecht *et al.*,[18] our findings implicate that aberrant lipid profiles are common pathophysiology of AD regardless of age and race. To our knowledge, the present study provides the first pediatric data on skin lipid profiles, microbiome, TEWL, and their relationships using an STS method, which is a non-invasive and reliable technique for obtaining human SC samples.[19,33,34]

It has been established that the total amount of SC CERs is reduced in the lesional and nonlesional skin of pediatric and adult AD patients compared to the skin of healthy subjects.[35] In that study, the levels of long-chain CERs (> 42 carbon atoms) were reduced in AD patients


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aged 4-40 years along with an increase in the levels of short-chain CERs (< 42 carbon atoms). Unfortunately, examining the total number of carbon atoms of CERs alone has limitations in evaluating the lengths and degrees of unsaturation in FA chains.[35] In contrast, our study demonstrated aberrant SC lipid profiles with carbon chain lengths of FA for the first time in children with AD. In another study involving pediatric AD patients, the amounts of specific CER subclasses, such as CERs with sphingosine N-acylated with α-hydroxy FA (AS-CERs) and NS-CERs, were elevated in Chinese children.[36] In our study, there were no differences in the amounts of AS-CERs and NS-CERs between the AD and control groups (data not shown), while the levels of total CERs and EOS-CERs were decreased in AD patients. The reason for this discrepancy might be the differences in characteristics of the study populations, including race, age, and AD severity. Subjects in the Chinese study were younger and showed higher SCORAD scores compared to those in the present study. Also, the concentration of skin EOS-CERs showed a tendency to increase in AD patients compared to healthy controls in that Chinese study, as opposed to our findings and the results from a previous study involving Caucasian adult AD patients.[16] A detailed evaluation of total serum IgE levels and allergic sensitization was not performed in the present study. Further research is needed on SC lipid profiles in AD according to patient characteristics including extrinsic or intrinsic AD.

As shown in the control group of the present study, the most prevalent SC CERs contained long saturated FA hydrocarbon chains between C24-26, and the length of FA chains affected skin permeability.[37] In contrast, the chain length of FAs in phospholipids such as SMs and LPCs as well as CERs was reduced in the lesional and nonlesional skin of children with AD, similar to adult patients.[18,19] The effects of FA chain length of SC lipids on membrane architecture and permeability indicate the importance of the disrupted skin barriers associated with the lipid layer abnormalities in the pathophysiology of AD. Unlike CERs with LCFAs, CERs with SCFAs (C16-18) do not provide effective interaction with other lipid chains and barrier functions despite the same polar head groups and hydrogen bonding abilities.[1] We also found that the levels of monounsaturated or polyunsaturated lipids were increased in AD skin regardless of chain length, in accordance with the results of a previous study.[38] Interestingly, in the present study, unsaturated FAs showed a similar trend to SCFAs in terms of the association between SC lipids and microbiomes. This supports the finding that an increased degree of unsaturation leads to skin barrier dysfunction by lowering the packing density in the lipid organization.[38]

It has been reported that Th2 immunity is responsible for altered epidermal lipid profiles, which include decreased levels of FFAs and EOS-CERs and increased levels of unsaturated FFAs.[15,16] Our previous study showed that Th2 immune activation downregulated the expression of FA elongases 3 and 6 (ELOVL3 and ELOVL6), leading to an increased proportion of short-chain species in epidermal lipid classes with a decrease in the proportion of long-chain species in AD skin.[19] In that study, signal transducer and activator of transcription 6 (STAT6) silencing prevented IL-4/IL-13–driven changes in human keratinocytes, indicating that Th2 cytokines inhibited ELOVL3 and ELOVL6 expression in a STAT6-dependent way.[19] Th2 cytokines also decreased the mRNA levels of elongase 1, acid-sphingomyelinase, and β-glucocerebrosidase, which were involved in the synthesis of very-long-chain FFAs and CERs.[39] Th2 immunity and subsequent changes in elongases are also postulated as the cause of altered skin lipids in our pediatric AD patients.

It has been suggested that commensal bacteria such as *S. epidermidis* and *S. hominis* can prevent cutaneous dysbiosis by inhibiting *S. aureus* colonization, which is a major risk factor for


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skin microbial dysbiosis.[40,41] Skin colonization with commensal *Staphylococcus* during early infancy has also been reported to prevent AD development at 12 months of age.[42] In recent studies in Germany, the levels of long-chain unsaturated FFAs in CERs were associated with increases in lipophilic *Propionibacteria* and *Corynebacterium* in the skin of adult AD patients.[18,24] Additionally, *FLG*-deficient subjects showed decreased abundance of Proteobacteria and the genera *Acinetobacter, Enhydrobacter*, and *Microvirgula* and increased proportions of Firmicutes, *Propionibacterium*, and *Staphylococcus*.[18] Similarly, our study showed a positive association of FA chain lengths of SC CERs and LPCs with the genus *Corynebacterium* and the phylum Proteobacteria as well as a negative association with the genus *Staphylococcus* and the phylum Firmicutes, supporting the role of SC lipids in these microbes, or vice versa. We also observed a positive correlation of FA chain lengths of SC CERs and LPCs with the phyla Actinobacteria, Bacteroidetes, and Fusobacteria and the genera *Streptococcus*, *Enhydrobacter*, *Micrococcus*, and *Bacteroides*. Of note, recent studies demonstrated the bactericidal activity of skin lipids, including sphingoid bases and FAs, against specific bacterial compositions as a part of innate immunity.[43,44] For example, short-chain C6-CERs exhibited antibacterial activity against *Neisseria meningitidis* and *N. gonorrhoeae,* while they were inactive against *Escherichia coli* and *S. aureus*.[44] This indicates that chain length, type of hydroxylation, and saturation of SC lipids contribute to the predominance of specific microbes in the skin. Conversely, skin microbial dysbiosis can result in aberrant lipid profiles in AD skin. It has been reported that *S. aureus* lipases support the penetration of *S. aureus* into hair follicles, but the lipases do not alter FA chain length.[45] However, *S. aureus* colonization in AD skin can lead to Th2 polarization and subsequent epidermal lipid abnormalities by inhibiting elongases.[5,12,46] Therefore, these findings indicate that epidermal lipid alterations are associated with microbial dysbiosis in children with AD, as in adults with AD.

In conclusion, AD skin in children, like adults, shows aberrant lipid profiles including an increase in SCFA-containing species of C18-NS-CERs, SMs, and LPCs along with a decrease in the proportion of corresponding long-chain species, and these alterations are associated with skin microbial dysbiosis and cutaneous barrier dysfunction. Children with AD may derive clinical benefits from future treatment options targeting aberrant lipid profiles or microbial imbalances, although further longitudinal cohort studies are needed to establish a causal relationship.

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