

Epidermal lipid composition, barrier integrity, and eczematous inflammation are associated with skin microbiome configuration



Hansjörg Baurecht, PhD,^{a*} Malte C. Rühlemann, MSc,^{b*} Elke Rodríguez, PhD,^a Frederieke Thielking, MD,^a Inken Harder, MSc,^a Anna-Sophie Erkens, MD,^a Dora Stölzl, MD,^a Eva Ellinghaus, PhD,^b Melanie Hotze, MSc,^a Wolfgang Lieb, MD, MSc,^c Sheng Wang, PhD,^d Femke-Anouska Heinsen-Groth, PhD,^b Andre Franke, PhD,^b and Stephan Weidinger, MD^a Kiel, Germany, and Hangzhou, China

Background: Genomic approaches have revealed characteristic site specificities of skin bacterial community structures. In addition, in children with atopic dermatitis (AD), characteristic shifts were described at creases and, in particular, during flares, which have been postulated to mirror disturbed skin barrier function, cutaneous inflammation, or both.

Objective: We sought to comprehensively analyze microbial configurations in patients with AD across body sites and to explore the effect of distinct abnormalities of epidermal barrier function.

Methods: The skin microbiome was determined by using bacterial 16S rRNA sequencing at 4 nonlesional body sites, as well as acute and chronic lesions of 10 patients with AD and 10 healthy control subjects matched for age, sex, and filaggrin (*FLG*) mutation status. Nonlesional sampling sites were characterized for skin physiology parameters, including chromatography-based lipid profiling.

Results: Epidermal lipid composition, in particular levels of long-chain unsaturated free fatty acids, strongly correlated with bacterial composition, in particular *Propionibacteria* and *Corynebacteria* abundance. AD displayed a distinct community structure, with increased abundance and altered composition of staphylococcal species across body sites, the strongest loss of diversity and increase in *Staphylococcus aureus* seen on chronic lesions, and a progressive shift from nonlesional skin to acute and chronic lesions. *FLG*-deficient skin showed a distinct microbiome composition resembling in part the AD-related pattern.

Conclusion: Epidermal barrier integrity and function affect the skin microbiome composition. AD shows an altered microbial configuration across diverse body sites, which is most pronounced at sites of predilection and AD. Eczematous affection appears to be a more important determinant than body site. (J Allergy Clin Immunol 2018;141:1668-76.)

Key words: Atopic dermatitis, filaggrin, lipidome, microbiome

From ^athe Department of Dermatology and Allergy, University Hospital Schleswig-Holstein, Campus Kiel; ^bthe Institute of Clinical Molecular Biology, Christian Albrechts University of Kiel; ^cthe Institute of Epidemiology and Biobank PopGen, Christian-Albrechts-University of Kiel; and ^dthe College of Life Information Science and Instrument Engineering, Hangzhou Dianzi University, Hangzhou.

*These authors contributed equally to this work.

The project received infrastructure support through the DFG Clusters of Excellence “Inflammation at Interfaces” (grants EXC306 and EXC306/2) and was supported by the German Federal Ministry of Education and Research (BMBF) within the framework of the e:Med research and funding concept (sysINFLAME, grant 01ZX1306A).

Disclosure of potential conflict of interest: M. C. Rühlemann’s institution received grant DFG-CRC 1182 for this work. F.-A. Heinsen-Groth’s institution received grant 01ZX1306A from German Federal Ministry of Education and Research for this work. S. Weidinger’s institution received DFG Clusters of Excellence “Inflammation at Interfaces” (grants EXC306 and EXC306/2) and German Federal Ministry of Education and Research (BMBF) grants within the framework of the e:Med research and funding concept (sysINFLAME, grant 01ZX1306A) for this work. The rest of the authors declare that they have no relevant conflicts of interest.

Received for publication September 15, 2017; revised December 20, 2017; accepted for publication January 3, 2018.

Available online February 5, 2018.

Corresponding author: Stephan Weidinger, MD, Department of Dermatology and Allergy, University Hospital Schleswig-Holstein, Campus Kiel, Rosalind-Franklin Str. 7, 24105 Kiel, Germany. E-mail: sweidinger@dermatology.uni-kiel.de.

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0091-6749/\$36.00

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<https://doi.org/10.1016/j.jaci.2018.01.019>

The skin shows strong topographic variations and spatial differences in terms of cellular makeup and molecular characteristics, which, under normal physiologic conditions, create a distinct pattern of microenvironments that differ in humidity, pH, temperature, antimicrobial peptide composition, and lipid content.^{1,2} Landmark genomic studies in healthy subjects showed that the composition of microbial communities on the skin surface displays marked regional differences, which have been postulated to be driven by differences in skin physiology.³⁻⁶ Microbiota can affect epidermal barrier function⁷ and cutaneous immunity.⁸ Thus the mutualistic relationship between physical, chemical, and immunologic features of the skin barrier and microbial populations can create pathogenicity islands that, under certain pathologic conditions, underlie the remarkable site specificities of many skin diseases.

A paradigmatic skin disorder with such a characteristic distribution of lesions and a postulated disturbance of the balance between host and microbes is atopic dermatitis (AD).^{9,10} Despite induction of antimicrobial peptide in AD lesions,¹⁰ colonization with *Staphylococcus aureus* is found in the vast majority of patients.¹¹ With the help of sequencing-based surveys, a reduced overall diversity of microbial communities in favor of *S aureus* was observed at AD predilection sites, in particular during disease flares.¹² However, it is unclear whether a shifted skin microbiome

Abbreviations used

AD:	Atopic dermatitis
Af:	Antecubital fossa
AS:	α -Hydroxy fatty acid/sphingosine base
CoNS:	Coagulase-negative staphylococci
Ef:	Extensor forearm
FDR:	False discovery rate
FFA:	Free fatty acid
FLG:	Filaggrin
GLM:	Generalized linear model
OTU:	Operational taxonomic unit
SFC-MS/MS:	Supercritical fluid chromatography mass spectrometry
TEWL:	Transepidermal water loss
Vf:	Volar forearm

is a general feature in patients with AD or restricted to certain sites if the lesional microbiome signatures are dependent on the acuity of inflammation and how defined insults to epidermal barrier function and integrity affect the microbiome.

The best characterized cause for a generalized skin barrier impairment is an inherited deficiency of the epidermal structural protein filaggrin (FLG) because of a single null mutation, as seen in up to 10% of the population and up to a third of patients with AD.⁹ Apart from a decreased integrity of the stratum corneum, FLG deficiency has also been suggested to affect lipid formation¹³ and enhance *S aureus* growth.¹⁴ A recent study further suggested a lower relative abundance of gram-positive anaerobe cocci in *FLG*^{-/-} skin,¹⁵ but that study examined a single location only and compared “extremes” (ie, subjects with a complete FLG deficiency with ichthyosis vulgaris). Here we set out to gain more insights into the effect of *FLG* haploinsufficiency, epidermal physiology, and incident AD on skin microbiome configuration at different body sites and through disease stages.

METHODS

Study population

For this study, 4 groups of German adults from the PopGen population-based biobank¹⁶ were invited to participate in a follow-up examination: (1) patients with AD carrying a single *FLG* mutation (AD FLGmut), (2) patients with AD without *FLG* mutations (AD FLGwt), (3) patients with no history of chronic skin or allergic disorders carrying a single *FLG* mutation (control FLGmut), and (4) patients with no history of chronic skin or allergic disorders without *FLG* mutations (control FLGwt). Information on the *FLG* genotype was available from prior studies.¹⁷ Proband characteristics are described in Table E1 in this article's Online Repository at www.jacionline.org. The follow-up examination included a skin examination by an experienced dermatologist blinded to the genotype. AD was diagnosed by using American Academy of Dermatology diagnostic criteria.¹⁸ For the current analysis, 5 subjects per group were selected and matched by age and sex. Patients with AD selected had to display at least 1 unaffected antecubital crease. None of the participants had received systemic immunosuppressants or systemic antibiotics in the preceding 3 months. All participants were asked to avoid bathing and application of any topical agent 24 hours before the examination visit. The study was approved by the ethics review board of the Medical Faculty of the University Kiel, Germany, and written informed consent was obtained from all study participants.

Sampling

At sampling sites, skin pH was measured with a Skin-pH-Meter (HI-99181/HI-1414D; Hanna Instruments, Vöhringen, Germany), and transepidermal water loss (TEWL) was measured with the Tewameter TM 300

(Courage + Khazaka Electronic GmbH, Cologne, Germany), according to the manufacturer's instructions. The mean of 3 measurements was used for analysis.

Skin swabs were taken from 4 different nonlesional sites (forehead, antecubital fossa [Af], volar forearm [Vf], and extensor forearm [Ef]) and from a chronic skin lesion in patients with AD. In 6 of the patients with AD, an additional swab was taken from an acute lesion defined as a new lesion of less than 24 hours' duration without clinical signs of lichenification. Skin swabs were collected by swabbing a 4-cm² area with sterile Catch-All Sample Collection Swabs (Epicentre Biotechnologies, Madison, Wis) soaked in sterile specimen collection fluid (SCF-1) solution, as previously described.¹⁹ As negative controls, we took 2 swabs exposed only to ambient air. DNA was extracted with the PowerSoil DNA Isolation Kit (MoBio Laboratories, Carlsbad, Calif), as described previously.²⁰

To harvest stratum corneum lipids, 4 tape strips per site were taken by using D-Squame discs (CuDerm, Dallas, Tex), as described previously.²¹ Tapes were placed in sterile 1.5-mL Eppendorf tubes (Eppendorf, Hamburg, Germany) and stored at -80°C immediately.

Processing of bacterial 16S rRNA sequenced data

Bacterial DNA was amplified, and the 16S rRNA gene was sequenced, as previously described (see the Methods section in this article's Online Repository at www.jacionline.org for details).²² Briefly, the amplicon-specific hypervariable regions V1 and V2 of the bacterial 16S rRNA gene were sequenced on the Illumina MiSeq platform (Illumina, San Diego, Calif). After demultiplexing and quality trimming (<https://github.com/najoshi/sickle>), forward and reverse sequences were combined by using VSEARCH.²³ Quality control and chimera filtering were carried out with the FastX Toolkit (http://hannonlab.cshl.edu/fastx_toolkit/) and UCHIME algorithm.²⁴ Cleaned FastA sequences were combined and clustered into operational taxonomic units (OTUs) by using the UPARSE algorithm.²⁵ For each sample, a subset of 10,000 random reads was picked to construct the OTU abundance table. Taxonomic classification from the phylum to genus level for the same 10,000 reads was performed with the RDP classifier and the Greengenes database.²⁶ Classification of OTUs to *Staphylococcus* species (98% similarity threshold) was performed by comparing against the EzBioCloud database (<http://www.ezbiocloud.net>).²⁷ Taxa with more than 90% of their counts within the first 5% of the count range were excluded. From the remaining taxa, a taxon was considered a “colonist” of a body site if more than 0.1% of the reads per sample mapped to the recovered genome.

Lipid analysis

Three hundred forty-eight ceramides, 12 free fatty acids (FFAs), and cholesterol sulfate were semiquantitatively measured at 3 body sites by using a Waters UPC2/Sciex QTrap 550 mass spectrometer supercritical fluid chromatography mass spectrometry (SFC-MS/MS) system (Waters Corporation, Milford, Mass/Sciex, Framingham, Mass) with the TrueMass Stratum Corneum Lipid Panel (Metabolon, Durham, NC). Details on lipid analysis are provided in the Methods section in this article's Online Repository.

Data analysis and statistics

Details on statistical analysis are presented in the Methods section in this article's Online Repository. Briefly, all analyses were carried out with R 3.3.2 software (www.R-project.org).²⁸ For analyses of α - and β -diversities of the skin microbiome, the vegan (version 2.4) package was used, differences between groups were evaluated by using the Wilcoxon test, and correlation of taxa on different levels were calculated by means of Spearman correlation. Association of individual skin bacterial traits was analyzed by using generalized linear models (GLMs) with appropriate link functions and variance estimations to account for increasing numbers of zeros and overdispersion. For taxa with exhibits with more zeros than would be allowed in GLMs, we applied hurdle models implemented in the pscl (version 1.4.9) package,²⁹ and for multivariate taxa analysis, we used the mvabund (version 3.12.3) package. Ratios of microbial taxa abundance were first log-transformed and then carried forward to statistical analysis.

TABLE I. Mean relative abundance of genera present with at least 0.5% in healthy subjects in at least 3 sites

Phylum/genus	Af	Forehead	Ef	Vf	P value, Friedman test
Actinobacteria	36.7%	50.2%	44.7%	44.2%	.0327
<i>Propionibacterium</i>	19.0%	38.9%	26.1%	25.1%	.0062
<i>Corynebacterium</i>	9.8%	6.8%	10.6%	11.0%	.1968
<i>Micrococcus</i>	1.5%	0.9%	2.8%	2.1%	.0576
<i>Kocuria</i>	1.0%	0.6%	1.1%	0.9%	.2379
<i>Actinomyces</i>	0.7%	0.8%	0.7%	0.4%	.8964
<i>Rothia</i>	0.6%	1.0%	1.1%	0.3%	.0079
Firmicutes	45.0%	30.8%	36.3%	37.7%	.2933
<i>Staphylococcus</i>	31.3%	15.5%	16.8%	22.3%	.1524
<i>Streptococcus</i>	5.5%	5.6%	7.6%	3.8%	.1870
<i>Anaerococcus</i>	1.2%	1.6%	2.4%	2.7%	.1131
<i>Finegoldia</i>	1.6%	0.8%	1.8%	2.7%	.0560
<i>Veillonella</i>	1.1%	1.3%	1.4%	0.6%	.4618
<i>Lactobacillus</i>	1.0%	0.3%	1.4%	1.2%	.1576
<i>Peptoniphilus</i>	0.5%	0.4%	0.7%	1.1%	.7006
Proteobacteria	12.3%	14.9%	14.2%	13.4%	.5164
<i>Acinetobacter</i>	3.6%	2.3%	4.8%	4.5%	.5164
<i>Haemophilus</i>	0.7%	1.9%	1.5%	0.8%	.1604
<i>Enhydrobacter</i>	1.6%	0.6%	1.3%	1.4%	.2530
<i>Neisseria</i>	0.7%	1.0%	0.7%	0.2%	.8192
<i>Microvirgula</i>	1.0%	1.2%	2.5%	1.7%	.7650
Bacteroidetes	4.8%	2.7%	3.7%	4.2%	.7819
<i>Prevotella</i>	1.0%	0.6%	1.0%	0.9%	.3227
<i>Chryseobacterium</i>	1.2%	0.6%	1.1%	1.3%	.7086
Fusobacteria	0.8%	1.0%	0.8%	0.2%	.8935
<i>Leptotrichia</i>	0.7%	0.6%	0.5%	0.03%	.8584

Integrative analysis was applied to estimate the influence of skin lipids on microbial composition by using sparse canonical correlation analysis implemented in the PMA package.^{30,31}

Gradual changes of microbial taxa from nonlesional over acute to chronic lesions are evaluated by using linear mixed models with the lme4 package (version 1.1.13).

RESULTS

Microbiome profiling

In the microbiome of healthy subjects, 5 prevalent bacterial phyla accounted for more than 99% of each subject's microbiota (Actinobacteria, Proteobacteria, Firmicutes Bacteroidetes, and Fusobacteria). Twenty-one genera were present in at least 3 sites with at least 0.5% abundance (Table I). Four of these genera (*Propionibacterium*, *Corynebacterium*, *Staphylococcus*, and *Streptococcus*) were present in all 10 healthy subjects with at least 1% abundance. Across sites, staphylococci showed the highest abundance (15.9% to 31.3%), with *Staphylococcus epidermidis* being the most prevalent *Staphylococcus* species (51.9% to 55.2%). Similarity analysis showed that Vf and Ef sites cluster together and are distinctly separated from the Af and the forehead.

FLG deficiency correlates with decreased bacterial diversity and a distinct bacterial colonization pattern

In healthy subjects, across the 4 sampling sites, FLG deficiency shows a consistent decrease of α -diversity (mean difference at Af, 0.43; Vf, 1.06; Ef, 0.39; and forehead, 0.22). Except for the

forehead, we also observed a lower genera richness, which was most pronounced at the AD predilection site Af (see Fig E1 in this article's Online Repository at www.jacionline.org). The similarity analysis for each site showed a clear separation of FLG-deficient from FLG-competent subjects. Across all sites, FLG-deficient subjects displayed a decreased abundance of the Proteobacteria genera *Acinetobacter*, *Enhydrobacter*, and *Microvirgula* (Af: 1.1% vs 11.1%, $P_{\text{false discovery rate [FDR]}} = .035$; Vf: 1.8% vs 13.4%, $P_{\text{FDR}} = .002$; and Ef: 2.5% vs 14.7%, $P_{\text{FDR}} = .002$; Fig 1). At the same time, FLG-deficient subjects displayed an increased abundance of *Propionibacterium* (Af: 23.3% vs 14.7%, $P = .020$; Vf: 32.1% vs 18.0%, $P = .010$; and Ef: 34.1% vs 18.0%, $P_{\text{FDR}} = 4.0 \times 10^{-4}$). Furthermore, there was a tendency toward increased Firmicutes abundance, in particular for the genus *Staphylococcus*. However, there was no specific association with *S aureus*, *S epidermidis*, or *Staphylococcus hominis* abundance.

We also investigated whether FLG deficiency facilitates unique bacteria colonization. subjects carrying an FLG mutation showed a consistently reduced abundance of the rare genus *Chryseobacterium* (Af: 0.06% vs 2.33%, $P_{\text{FDR}} = 8.9 \times 10^{-4}$; Vf: 0.33% vs 2.20%, $P_{\text{FDR}} = .049$; and Ef: 0.51% vs 1.65%, $P = .030$). *Chryseobacterium* is found most commonly in soil, water, and contaminated medical devices,³² is regarded an opportunistic pathogen, and has not been identified previously as a component of the skin microbiome.

The skin microbiome composition of healthy FLG-deficient subjects was more similar to that of patients with AD than healthy FLG-competent subjects, in particular at the Af (see Fig E2 in this article's Online Repository at www.jacionline.org).

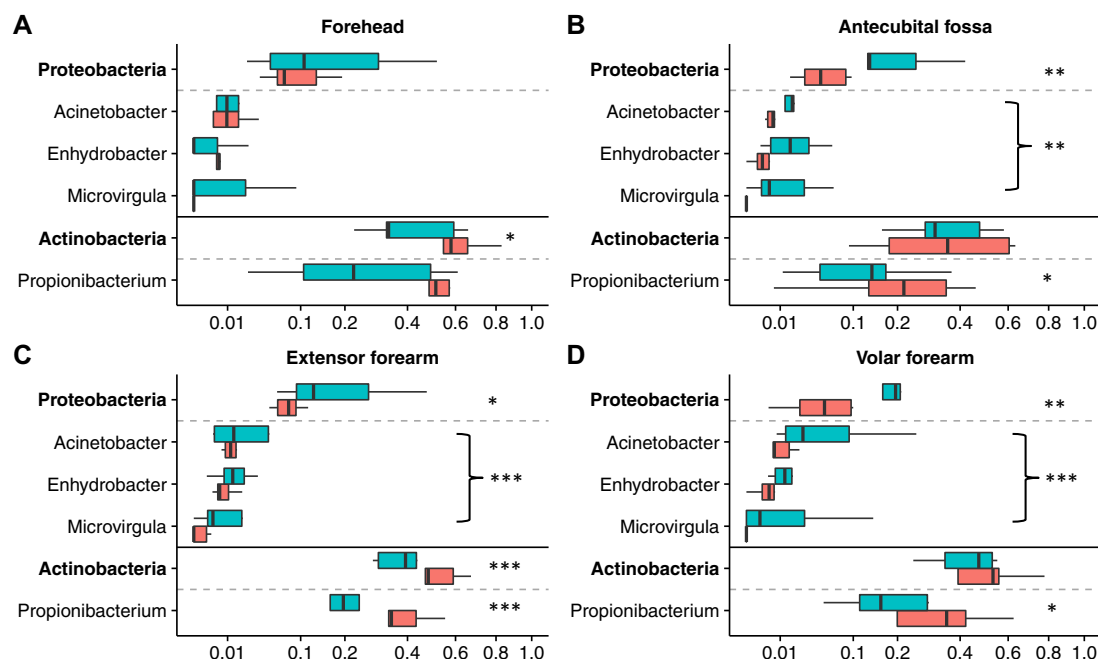


FIG 1. Effect of *FLG* deficiency on the skin microbiome. Proteobacteria and Actinobacteria genera showing differential abundance in *FLG*-competent and *FLG*-deficient subjects at the forehead (A), Af (B), Vf (C), and Ef (D). For better visualization, abundance is depicted on the square root scale. Red bars depict *FLG* mutation carriers, and blue bars depict non-*FLG* mutation carriers. Asterisks indicate significance obtained from the Wilcoxon test: * $P < .05$, ** $P < .005$, and *** $P < .0005$.

Epidermal lipid composition affects skin microbiome diversity and composition

Integrated analysis revealed a strong association between microbiome and lipidome composition ($P_{\text{FDR}} = .015$; Fig 2 and see Fig E3 in this article's Online Repository at www.jacionline.org). More specifically, the abundance of *Propionibacterium* and *Corynebacterium* showed a strongly positive correlation with levels of unsaturated long-chain FFAs, such as FA20:1, FA20:2, FA22:1, and FA24:1, and a negative correlation with saturated short-chain FFAs, such as FA16:0 and FA18:0 (Fig 2), particularly at the Af and Vf (see Fig E4 in this article's Online Repository at www.jacionline.org). Furthermore, at the AD predilection site Af, increasing levels of unsaturated long-chain FFAs correlated with decreased α -diversity (see Fig E5 in this article's Online Repository at www.jacionline.org), whereas increasing levels of α -hydroxy ceramide (AS) correlated with higher abundances of staphylococci ($r = 0.64$, $P_{\text{FDR}} = .030$; see Fig E4), in particular *S aureus* ($r = 0.57$, $P = .0088$). Interestingly, at the Af, levels of ceramides (α -hydroxy fatty acid/6-hydroxy-sphingosine base, AS, α -hydroxy fatty acid/phytosphingosine base, and nonhydroxy fatty acid/phytosphingosine base) and several FFAs (FA20:1, FA20:2, FA22:1, and FA24:1) were negatively correlated with abundance of the gram-negative Proteobacteria *Haemophilus* and *Neisseria* species and positively correlated with the abundance of the Actinobacteria and *Propionibacterium* and *Corynebacterium* species (see Fig E4). In line with previous studies,³³ levels of nonhydroxy ceramide subclasses NS (nonhydroxy fatty acid/sphingosine base) and NH (nonhydroxy fatty acid/6-hydroxy-sphingosine base) were altered in patients with AD, but no clear association between *FLG* deficiency and levels of a specific lipid species was observed (see Fig E6 in this article's

Online Repository at www.jacionline.org). Detailed information about the investigated skin lipid profiles species are presented in Table E2 in this article's Online Repository at www.jacionline.org.

Neither TEWL nor skin pH showed clear associations with bacterial community patterns. However, skin pH, in line with previous studies,³⁴ showed little variation between sites and subjects.

AD displays an increased *Staphylococcus* species abundance and altered *Staphylococcus* species composition across diverse body sites

In both patients with AD and control subjects, the most prevalent *Staphylococcus* species were *S epidermidis* and *S hominis*, which accounted for up to 32.4% and 7.9% and up to 14.6% and 10.9 of the microbiota%, respectively (Fig 3, B). In line with previous studies on healthy subjects,^{4,35,36} also in patients with AD, the bacterial composition was strongly dependent on the anatomic region (Fig 3, A).

Although diversity and genera richness were reduced at the Af but not at other sites (see Fig E7 in this article's Online Repository at www.jacionline.org), the bacterial configuration in patients with AD was clearly distinct from that of healthy subjects across body sites, including nonaffected and nonpredilection sites. In particular, across all sites, patients with AD showed decreased *Propionibacterium*, *Kocuria*, and *Chryseobacterium* and increased *Lactobacillus* species abundance (see Fig E8 in this article's Online Repository at www.jacionline.org). More specifically, in patients with AD, *Chryseobacterium* and *Kocuria* species were significantly reduced at the forehead ($P = .0210$

	Propioni- bacterium	Coryne- bacterium	Kocuria	Rothia	Staphylo- coccus	Strepto- coccus	Anaero- coccus	Fine-goldia	Acinetobacter	Haemo- philus	Neisseria	Chryseo- bacterium
AH	0.310	0.436	0.026	-0.291	-0.081	-0.087	0.207	0.322	0.424	-0.086	-0.430	0.634
AS	0.407	0.471	-0.033	-0.154	0.060	0.068	0.391	0.416	0.389	0.118	-0.231	0.387
AP	0.337	0.468	0.019	-0.302	-0.086	-0.084	0.224	0.343	0.452	-0.082	-0.452	0.671
NP	0.179	0.287	0.081	-0.263	-0.063	-0.121	0.126	0.233	0.307	-0.127	-0.350	0.487
FA16:0	-0.436	-0.555	0.067	0.260	0.089	0.010	-0.280	-0.379	-0.498	0.000	0.444	-0.701
FA18:0	-0.429	-0.552	0.054	0.272	0.090	0.023	-0.276	-0.381	-0.500	0.014	0.454	-0.710
FA18:2	0.206	0.095	-0.339	0.320	0.032	0.325	0.101	-0.039	-0.056	0.357	0.265	-0.233
FA20:1	0.586	0.529	-0.490	0.226	-0.023	0.415	0.336	0.226	0.290	0.464	0.021	0.209
FA20:2	0.631	0.622	-0.430	0.104	-0.049	0.343	0.372	0.313	0.404	0.387	-0.128	0.403
FA22:1	0.634	0.573	-0.529	0.244	-0.026	0.449	0.364	0.246	0.315	0.501	0.021	0.228
FA24:1	0.600	0.557	-0.472	0.189	-0.031	0.393	0.347	0.253	0.325	0.441	-0.025	0.268

≥0.8

-0.5≤r<-0.8

-0.3≤r<-0.5

-0.1≤r<-0.3

|r|<0.1

0.1≤r<0.3

0.3≤r<0.5

0.5≤r<0.8

≥0.8

FIG 2. Bacterial genera showing statistically significant correlations with skin lipid species across body sites. Overall correlation between bacterial abundance and levels of lipid species across all samples was calculated by using sparse canonical correlation analysis with feature selection. AH, α -Hydroxy fatty acid/6-hydroxy-sphingosine base; AS, α -hydroxy fatty acid/sphingosine base; AP, α -hydroxy fatty acid/phytosphingosine base; NP, nonhydroxy fatty acid/phytosphingosine base. FFAs are depicted as FAXX:Y, with XX indicating chain length and Y indicating the number of double bonds/degree of unsaturation.

and $P = .011$), Vf ($P = .0106$ and $P = .0122$), and Ef ($P_{\text{FDR}} = .049$ and $P = .0213$). In parallel, the abundance of the rare genus *Lactobacillus* was increased consistently across sites (forehead, $P_{\text{FDR}} = .034$; Af, $P = .1273$; Vf, $P = .0371$; and Ef, $P = .1077$; see Table E3 in this article's Online Repository at www.jacionline.org).

Furthermore, patients with AD exhibited a considerably greater staphylococcal abundance (forehead, 27.1% vs 15.5%; Af, 56.7% vs 31.3%; Vf, 24.5% vs 22.3%; and Ef, 21.0% vs 16.8%; see Table E3 and Fig E8). At the predilection site Af, but not at other sites, patients with AD displayed a considerably higher abundance of *S epidermidis* (32.4% vs 13.8%, $P_{\text{FDR}} = .049$), whereas the relative abundance of *S hominis* was decreased across all sites. Furthermore, *S aureus* was detected in all patients with AD and on all sites, with the greatest abundance at the Af (8.9%, $P_{\text{FDR}} = 1.1 \times 10^{-14}$), whereas it was only detectable in 4 of 10 control subjects with a very low abundance of less than 1%. Analysis of all pairwise ratios for the most prevalent taxa revealed that the AD-associated shift of microbial configuration was clearly driven by *S aureus* ($5.4 \times 10^{-6} < P < .02$), with a 31-, 85-, and 38-fold increase in the *S aureus/S epidermidis* ratio at the Af ($P_{\text{FDR}} = .015$), Vf ($P_{\text{FDR}} = 3.8 \times 10^{-4}$), and Ef ($P_{\text{FDR}} = .004$), respectively. Likewise, the *S aureus*/coagulase-negative staphylococci (CoNS) fold change at these 3 sites was 38, 88, and 30 times greater than that in healthy control subjects (Af, $P_{\text{FDR}} = .013$; Vf, $P_{\text{FDR}} = 3.8 \times 10^{-4}$; and Ef, $P_{\text{FDR}} = .008$; see Table E4 in this article's Online Repository at www.jacionline.org). The marked difference in the *S aureus*/CoNS ratio was independent from the *FLG* mutation status.

In a multivariate abundance analysis, AD disease severity was associated significantly with skin microbiome composition at the forehead ($P = .022$) and Ef ($P = .008$). In particular, higher disease severity was associated with increased abundance of the genus *Corynebacterium* (forehead: $r = 0.79$, $P = .0062$; Ef: $r = 0.88$, $P_{\text{FDR}} = .011$) and reduced abundance of the Proteobacteria and Acinetobacteria and *Microvirgula* species (forehead: $r = -0.73$, $P = .016$; Ef: $r = -0.81$, $P_{\text{FDR}} = .011$) but not with any prevalent *Staphylococcus* species.

A progressive shift of *Staphylococcus* species composition characterizes acute and chronic eczema

Both acute and chronic lesions showed markedly reduced bacterial diversity and genera richness that was independent of body site (see Fig E9 in this article's Online Repository at www.jacionline.org) and showed distinct clusters that were well separated from nonlesional sites in principal coordinate analysis (Fig 4, A). Thus the effect of inflammation appears to superimpose locoregional influences on microbiome composition. Furthermore, there was a clear distinction of the microbiome composition between acute and chronic lesions (Fig 4, A). Overall, the abundance of staphylococci increased from nonlesional skin samples (32.4%) over acute (46.7%) to chronic (57.3%) lesions, with a significant difference between chronic lesions and nonaffected skin ($P = .014$; Fig 4, B). In parallel, *S aureus* abundance showed a gradual and significant ($P_{\text{mixed model}} = .0174$) increase from nonlesional skin samples (5.3%) over acute (23.1%) to chronic lesions (37.1%), whereas the abundance of *S epidermidis* and CoNS gradually decreased (nonlesional, 17.2% and 27.1%; acute, 14.1% and 23.7%; and chronic, 12.3% and 20.2%; see Fig E10, A-C, in this article's Online Repository at www.jacionline.org). In particular, there was a significantly ($P_{\text{FDR}} = .027$) greater *S aureus* abundance on chronic lesions compared with nonlesional skin (37.1% vs 5.3%). *S aureus/S epidermidis* (and *S aureus*/CoNS) ratios increased almost exponentially from 1:5 (1:7) to 1:1 and 4:1 (2:1) at nonlesional sites and acute and chronic lesions ($P_{\text{FDR}} = .002$ and $P_{\text{FDR}} = .006$, Fig 5). We also observed a gradual decrease of the Actinobacteria and *Corynebacterium* and *Propionibacterium* species from nonlesional skin ($8.7\% \pm 1.5\%$ and $18.8\% \pm 3.1\%$) over acute ($8.2\% \pm 2.1\%$, $P = .31$; $10.8\% \pm 5.3\%$, $P = .22$) to chronic lesions ($6.3\% \pm 1.0\%$, $P = .06$; $10.4\% \pm 2.9\%$, $P = .009$; Fig 4, B, and see Fig E10, D and E). Chronic lesions further showed a significant reduction of the phyla Bacteroidetes ($P_{\text{FDR}} = .027$) and Fusobacteria ($P = .013$; see Fig E10, F and G).

Three of the patients with AD reported use of lower-strength to midstrength topical corticosteroids at chronic lesional skin within 5 days of sampling. To exclude a potential bias, we carried out a

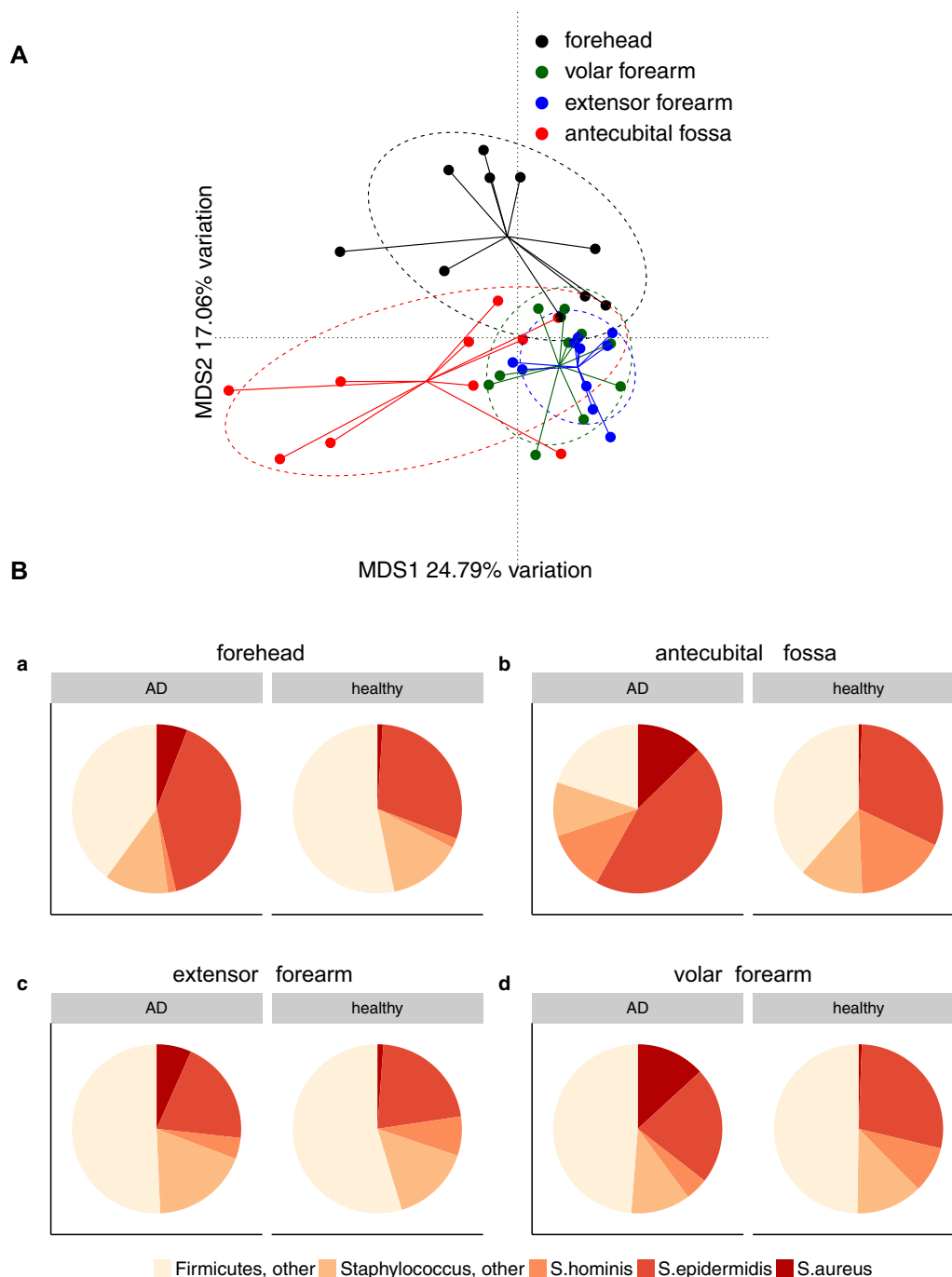


FIG 3. Site specificity of bacterial community composition in patients with AD. **A**, Based on the square root of the Bray-Curtis dissimilarities in principal coordinate analysis, the Vf and Ef clustered together but were distinct from the Af and forehead. **B**, The abundance of the most prevalent Firmicutes genera is strongly dependent on body site and shows significant differences between patients with AD and healthy control subjects.

sensitivity analysis using the Welch test, which showed no evidence of a significant effect of this treatment on diversity and microbial composition.

DISCUSSION

Although it has long been known that patients with AD show an increased cutaneous infectivity, that AD lesions are typically

colonized with *S aureus*,³⁷ and that *S aureus*-derived toxins have the capacity to exacerbate disease activity,³⁸ the role of microbes at large and their interplay with skin physiology in the pathogenesis of AD is still insufficiently understood. To gain deeper insight into the composition of skin microbiome in patients with AD and its interrelation with structural and physiochemical barrier functions, we examined a cohort of carefully matched and

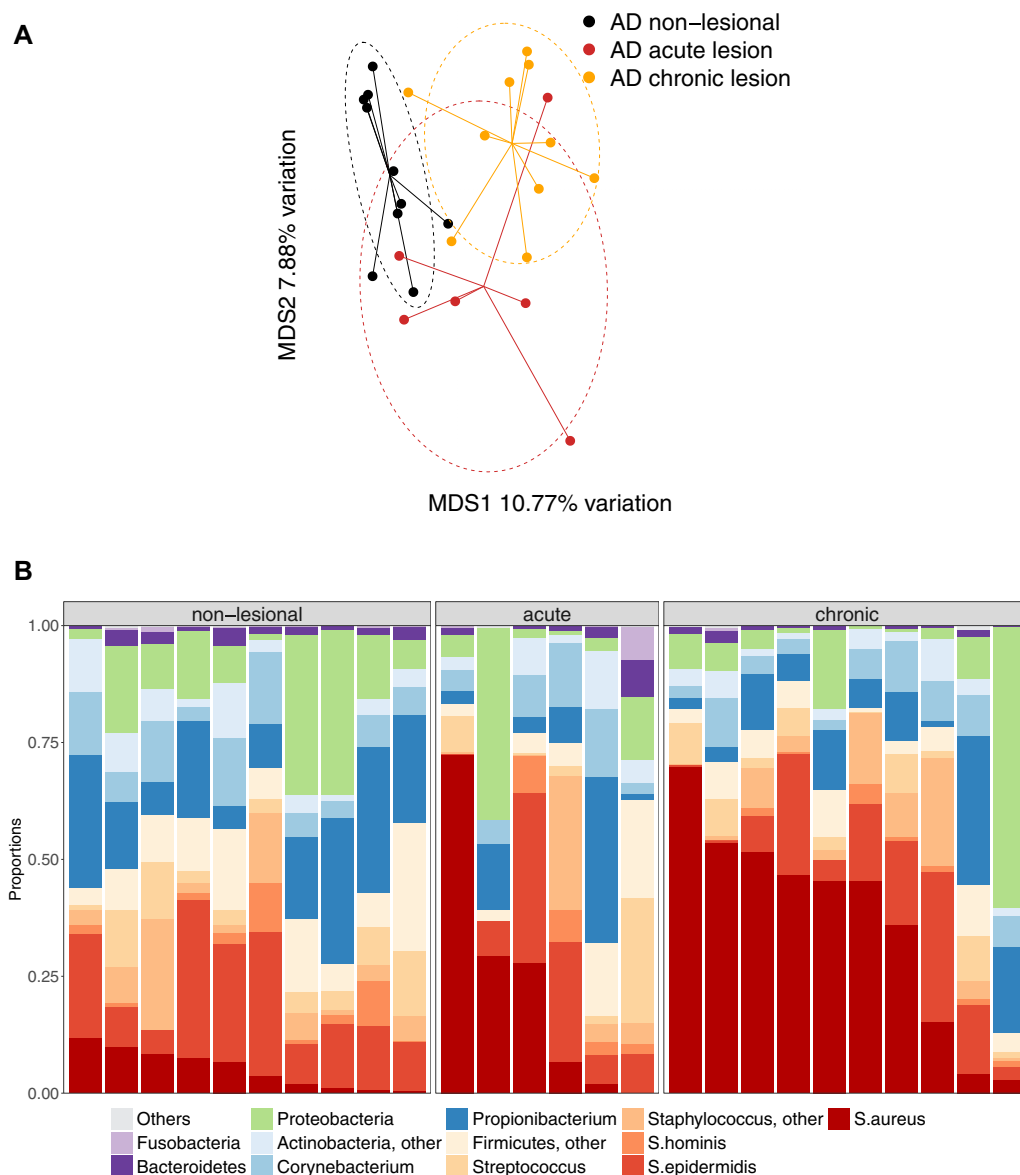


FIG 4. Affection by AD strongly affects bacterial composition. **A**, Principal component analysis showed distinct clustering of samples from nonlesional skin, acute AD lesions, and chronic AD lesions, irrespective of body site. **B**, Affection of skin by acute or chronic AD correlates with marked differences of the relative abundance of major phyla, genera, and *Staphylococcus* species, with the most pronounced shifts found on chronic lesions. The bar chart shows abundance at the individual level. *Non-lesional* depicts the average of the nonlesional samples from the 4 body sites: forehead, Af, Vf, and Ef.

comprehensively characterized patients with AD and healthy control subjects using 16S rRNA skin samples from different body sites, as well as from acute and chronic lesions.

For the first time, our study provides direct evidence for a strong effect of epidermal lipids on bacterial colonization, which presumably contributes to the marked site specificities of the skin microbiome. In particular, epidermal lipid composition correlated strongly with bacterial diversity and composition at AD predilection sites. Higher levels of long-chain unsaturated fatty acids were associated significantly with increased abundance of the lipophilic *Propionibacteria* and *Corynebacteria*,

which lack a fatty acid synthase³⁹ and require an exogenous source of FFAs.^{35,39,40} Furthermore, *S aureus* abundance was associated with higher levels of the ceramide AS, which was previously shown to be increased in AD skin, particularly in lesions.³³ In contrast, TEWL, which is often used as a proxy for epidermal barrier function, is not correlated directly with characteristics of the skin microbiome. In a previous study only correlation of total sebum from the cheeks with microbial composition and diversity was reported, which was investigated in healthy women from the Whitefield area of Bangalore city (India).⁴¹

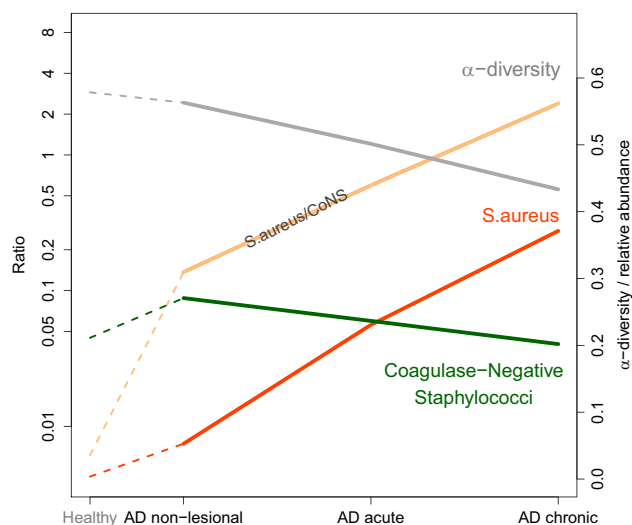


FIG 5. Hypothesized progression of bacterial composition from nonlesional to acute and chronic lesional skin in patients with AD. The *Staphylococcus* species line depicts *Staphylococcus* ratios, with the log-scaled y-axis on the left-hand side. Red and green lines indicate the *S aureus* and CoNS abundances with the corresponding y-axis on the right-hand side. The gray line shows the normalized α -diversity with the corresponding y-axis on the right-hand side. Dotted lines depict connections to the respective mean value of healthy subjects as a reference for interpretation.

Furthermore, our data show that an inherited deficiency of the epidermal structural protein FLG is associated with marked shifts in the composition of microbial communities, regardless of body site. FLG deficiency caused by inheritance of a single *FLG* mutation affects as much as 10% of the general population, which have generalized skin dryness caused by impaired formation of the stratum corneum and are at a greatly increased risk of AD.⁴²

Across body sites, FLG-deficient skin showed a reduced diversity and richness, increased abundance of Firmicutes, and decreased abundance of Proteobacterium, in particular the genus *Acinetobacter*, which is capable of suppressing the cytokine response to *S aureus*,⁴³ the signature microbe of AD. Furthermore, it showed an increased abundance of Actinobacteria, in particular *Propionibacterium* species, which have been reported to be underrepresented in patients with psoriasis,⁴⁴ another common inflammatory skin disease with largely opposing immune deviations compared with patients with AD.⁴⁵

Although most pronounced at disease predilection sites, in patients with AD, we observed an increased relative abundance of staphylococci, in particular *S aureus*, and an altered *Staphylococcus* species composition across nonaffected body sites, indicating that this is a general feature of AD not restricted to sites affected by eczema. AD is characterized by generalized abnormalities of skin barrier function with broad keratinocyte differentiation defects, inadequate regulation of antimicrobial peptide expression, compromised innate sensing, and considerable immune activation,^{46,47} which can contribute to such alterations of cutaneous colonization patterns. However, although these AD-related changes can drive microbial alterations, altered microbes might in turn play a role in disease-related immunologic changes⁴⁸ or likely both. Future studies with larger cohorts and longitudinal collection of samples at preclinical stages of disease will be required to better understand mutual influences.

A previous study reported an increase in the proportion of *Staphylococcus* species sequences, particularly *S aureus*, during AD flares and with increased general AD disease severity.¹² However, this observation was made only for predilection sites in children with moderate-to-severe AD (ie, at sites repeatedly affected by eczema). Although we found a strongly increased *Staphylococcus* species abundance at eczematous sites and, in particular, on chronic lesions, we did not observe associations between staphylococcal abundance and disease severity. However, across sites, we observed an association between AD severity and abundance of the genus *Corynebacterium* and the phylum Proteobacteria. Considerably more striking was the observation that eczematous skin changes appear to have a dramatically stronger influence on bacterial diversity and composition than, for example, the body site, underlining the important role of cutaneous immunity on microbial communities,⁴⁹ and that the alterations of bacterial community structures are more pronounced on chronic than acute lesions.

In conclusion, our data show that the molecular composition and function of the epidermis affect skin microbiome composition; that AD shows characteristic alterations, particularly regarding staphylococcal abundance and composition at predilection sites; and that the cutaneous inflammatory milieu and its acuity in eczema lesions superimpose site specificities.

However, it is important to note that the resulting sample size was relatively small and that we conducted many analyses because of the population-based and balanced design. Therefore *P* values need to be interpreted cautiously, and it is premature to draw strong conclusions. However, even at this small sample size, we were still able to identify significant microbiome differences between groups and disease states and identify differential abundant taxa after multiple testing corrections. Furthermore, we report correlations, and cause or effect still needs to be delineated. One approach to work out the effect of specific skin pathologies on the skin microbiota and *vice versa* and to gather evidence supporting causation will be to examine other types of eczema and inflammatory skin diseases.

Key messages

- Epidermal lipid composition affects bacterial community configuration.
- FLG deficiency correlates with decreased bacterial diversity and distinct bacterial colonization pattern.
- AD shows an altered skin microbiome configuration also at nonaffected and nonpredilection sites.
- The presence and chronicity of eczema appear to be more important determinants of skin microbiome configuration than body site.

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METHODS

Study population and sample collection

In addition to skin examination by an experienced dermatologist, the follow-up examination included a questionnaire on medical history. At examination, disease severity of the selected patients with AD was assessed quantitatively by using the SCORAD score.

All skin biophysical measurements, such as pH and TEWL, were done in standardized environmental conditions (room temperature, 22°C to 25°C; humidity levels, 30% to 35%) by the same investigator, according to international guidelines,^{E1} to minimize the influence of exogenous, environmental, and instrument-related factors.

Skin microbiota was collected by firmly swabbing a 4-cm² skin area for at least 30 seconds with sterile Catch-All Sample Collection Swabs (Epicentre Biotechnologies, Madison, Wis) soaked in sterile SCF-1 solution (50 mmol/L Tris buffer, 1 mmol/L EDTA, and 0.5% Tween-20 [pH 8.0]).

Stratum corneum lipids were obtained by attaching circular adhesive tape strips (3.8 cm²; D-Squame) to each sampled skin and pressed for 5 seconds of constant pressure (225 g/cm²) by using a D-Squame Pressure Instrument D500 (CuDerm).

Processing of bacterial 16S rRNA sequenced data

16S rRNA gene libraries were generated by using PCR from purified genomic DNA with primers 27F and 338R, targeting the hypervariable regions V1 and V2 of the 16S rRNA gene. Amplification and sequencing was performed by using a dual-indexing approach (8-nt on forward and reverse primers), as described by Kozich et al,^{E2} on the Illumina MiSeq platform generating 2 × 300-bp reads.

After demultiplexing, which is based on zero mismatches to the barcode sequence, reads were quality trimmed toward the 3'- and 5'-ends by using a sliding window of length of 0.1 times read length and cuts when the quality decreases to less than an average quality within the window of 20 (<https://github.com/najoshi/sickle>). Then forward and reverse sequences were combined into a single sequence by using overlap between the paired-end reads (minimum length, 280; maximum length, 350) with VSEARCH (<https://github.com/torognes/vsearch/>).^{E3} Quality control was performed with the FastX Toolkit (http://hannonlab.cshl.edu/fastx_toolkit/), and chimera filtering was done with the UCHIME algorithm (http://drive5.com/usearch/manual/uchime_algo.html) implemented in USEARCH software.^{E4} The cleaned FASTA sequences from all samples were combined, and sequences were clustered into OTUs at a similarity level of 97% by using the UPARSE algorithm implemented in the USEARCH software (http://www.drive5.com/usearch/manual/cmd_cluster_otus.html).^{E5} For each sample, a subset of 10,000 random reads was picked to construct the OTU abundance table. Taxonomic classification from the phylum to genus level for the same 10,000 reads was performed with the RDP classifier and the Greengenes database.^{E6} Classification of OTUs to *Staphylococcus* species (98% similarity threshold) was performed by comparing against the EzBioCloud taxonomic database (<http://www.ezbiocloud.net>).^{E7}

Lipid analysis

At 3 body sites (Af, forehead, and Vf), levels of 348 ceramides, 12 FFAs, and cholesterol sulfate were determined by using SFC-MS/MS with the TrueMass Stratum Corneum Lipid Panel (Metabolon). To this end, all D-Squame disks were extracted together with 4 quality control samples and using a polar and nonpolar organic solvent after addition of a known amount of surrogate standard solution: C16 ceramide-d31 ([S{C18}16:0]-d31), cis-10-heptadecenoic acid (FA17:1n7), and cholesteryl sulfate-d7. The organic extracts are combined and evaporated to dryness. The dried extract is reconstituted, and an aliquot is analyzed on a Waters UPC2/Sciex QTrap 5500 mass spectrometer SFC-MS/MS system in MRM mode by using characteristic parent-fragment mass transitions for each analyte trace.

The semiquantitative determination of individual analytes is based on their peak area comparison with the peak areas of their corresponding surrogate standards for which concentrations are known. C16 ceramide-d31 is used as a surrogate standard for all ceramides, all fatty acids are referenced to

cis-10-heptadecenoic acid, and cholesteryl sulfate is referenced to cholesteryl sulfate d7. Concentrations are given in picomoles per disk for individual analytes, as well as each lipid class. Additionally, the percentage composition of individual ceramide subtypes of the ceramide fraction is listed for each sample.

Data analysis and statistics

All data are presented as means ± SEMs, unless otherwise indicated. β-Diversity based on genus-level composition was computed by using the square root-transformed Bray-Curtis dissimilarity. Constrained principal coordinate analysis (nonmetric multidimensional scaling) on community ordination was performed by using the capscale function in vegan (version 2.4). Thereby either categorical data or continuous variables are linearly correlated with the coordinates of the microbial communities, and model selection is based on the Akaike information criterion provided by the standard R step function. Significance testing is carried out by means of permutation.

The Wilcoxon test for independent and dependent samples was applied to evaluate differences in α-diversities and relative abundance of individual skin bacterial traits on different taxa levels between groups at the same site or between sites within groups. Correlations of taxa of different levels (eg, phylum and genus) were calculated by using the Spearman correlation coefficient.

Additionally, multivariate and univariate association testing of the abundance of individual skin bacterial traits on different taxa levels with AD, *FLG* halpplinsufficiency, and biophysical parameters was carried out by using GLMs. For prevalent taxa in human skin with almost no zeros (eg, on the phylum level), we applied GLM with a Poisson distribution and a log link with correction for overdispersion by using robust sandwich covariance estimation with the sandwich package.^{E8,E9} For bacteria of low abundance, which are characterized by an increasing number of zeros at lower taxonomic levels and a right-skewed distribution, often with a long tail, we selected a GLM with a negative binomial (negbin) distribution and a log link for the statistical analysis (glm.nb function of the MASS version 7.3 package). For taxa that exhibit more zero observations than would be allowed for the negative binomial model (especially on the genus or species level), we applied the 2-component hurdle model with a negbin distribution using the pscl (version 1.4.9) package.^{E10} Therefore the GLM framework was applied consistently from the phylum to genus level, whereas analysis of rare genera and species was supported with the hurdle model. Multivariate taxa analysis was carried out with the mvabund (version 3.12.3) package (function manyglm). Significance testing was carried out by using nonparametric bootstrapping in which bootstrap samples were obtained by residual resampling of the probability integral transformation residuals.^{E11}

Because the use of ratios can lead to a strong reduction in overall variance, we computed and tested all possible pair microbial taxa abundance ratios of the most prevalent microbes. Microbial taxa abundance ratios were log-transformed before computing the statistics, and their back-transformation on the original scale can be interpreted as a ratio fold change. Note that testing ratios between 2 microbial taxa A and B are independent of their order, as follows:

$$\log(A/B) = -\log(B/A),$$

which halves multiple testing burden.

For estimating the influence of skin lipids on microbiome composition, we applied the integrative analysis method of sparse canonical correlation analysis using the R package PMA.^{E12,E13} To this end, we standardized lipid, TEWL, and pH measurements by using

$$y_i = (x_i - \bar{x})/std(x),$$

whereas taxa counts were transformed by using the inverse hyperbolic sine transformation as follows:

$$y_i = \log(x_i + (x_i^2 + 1)^{0.5}).^{E14}$$

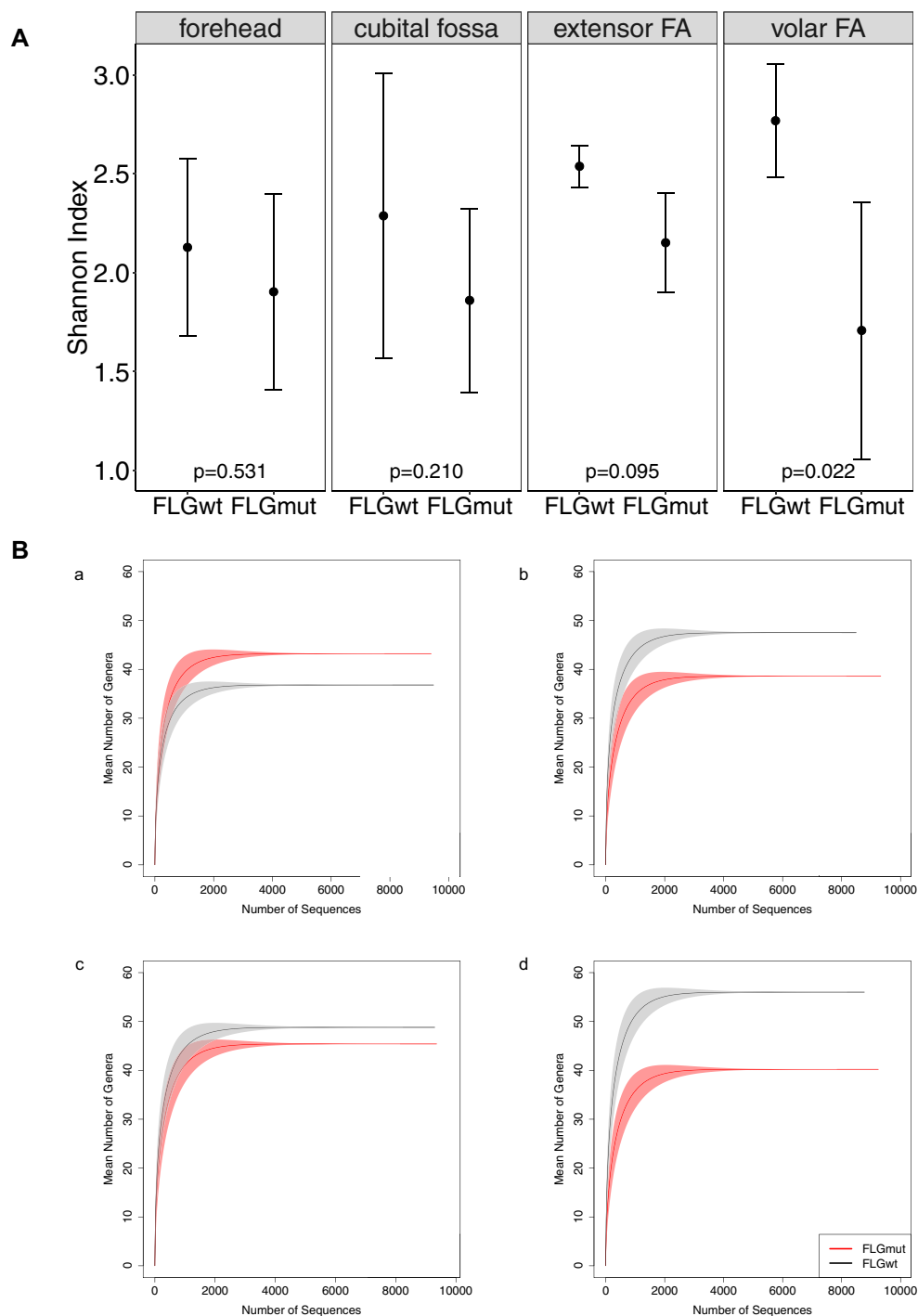
Significance was evaluated by using a permutation test.^{E15}

Gradual changes of microbial taxa from nonlesional over acute to chronic lesions are evaluated by using linear mixed models with random intercept for each subject by using the lme4 package (version 1.1.13).

All analyses have to be interpreted as exploratory, and the presented *P* values are unadjusted, if not otherwise stated. If adjustment for multiple testing was applied, the FDR for univariate analysis and the stepdown resampling procedure (mvabund package) for multivariate analysis were used, and respective *P* values are indicated.

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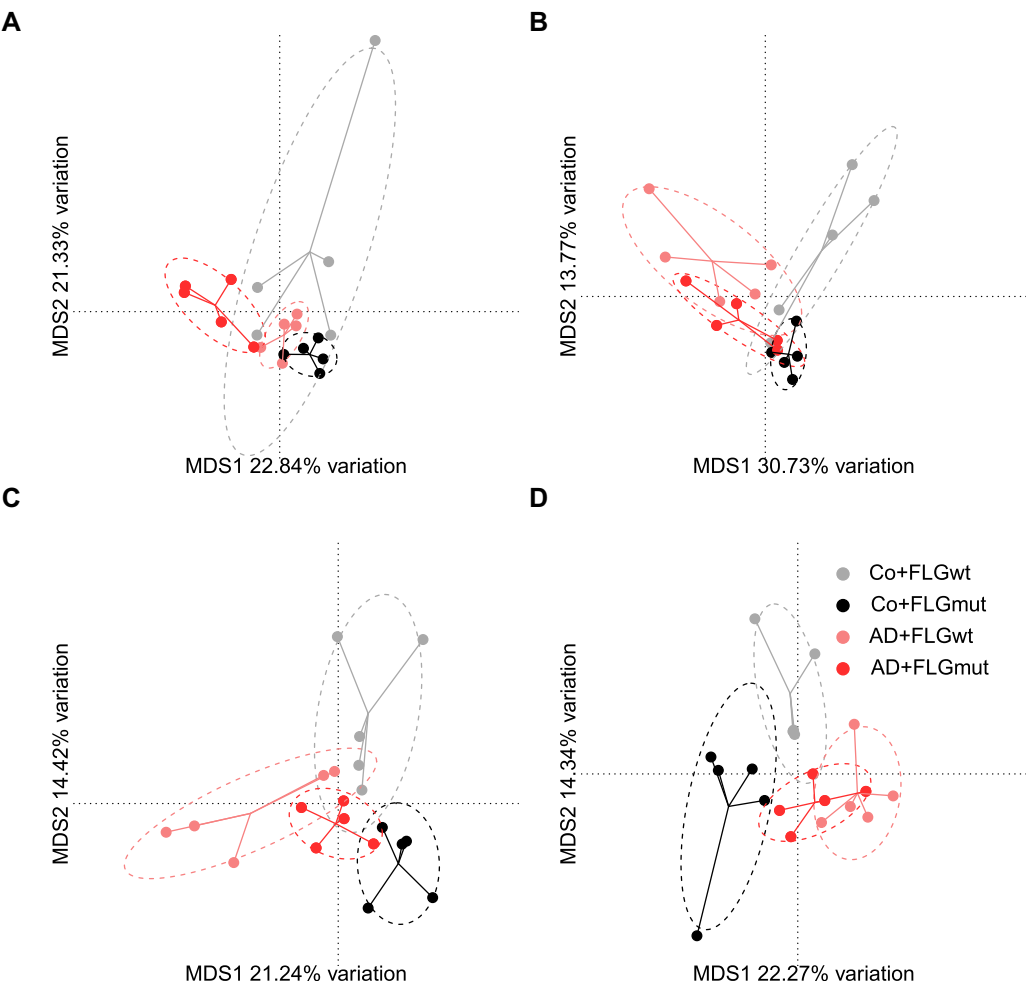


FIG E2. Bacterial community composition of *FLG*-deficient subjects is shifted toward the composition of patients with AD. Principal coordinates analysis was calculated based on the square root of the Bray-Curtis distance. **A**, Forehead; **B**, Af; **C**, Ef; and **D**, Vf.

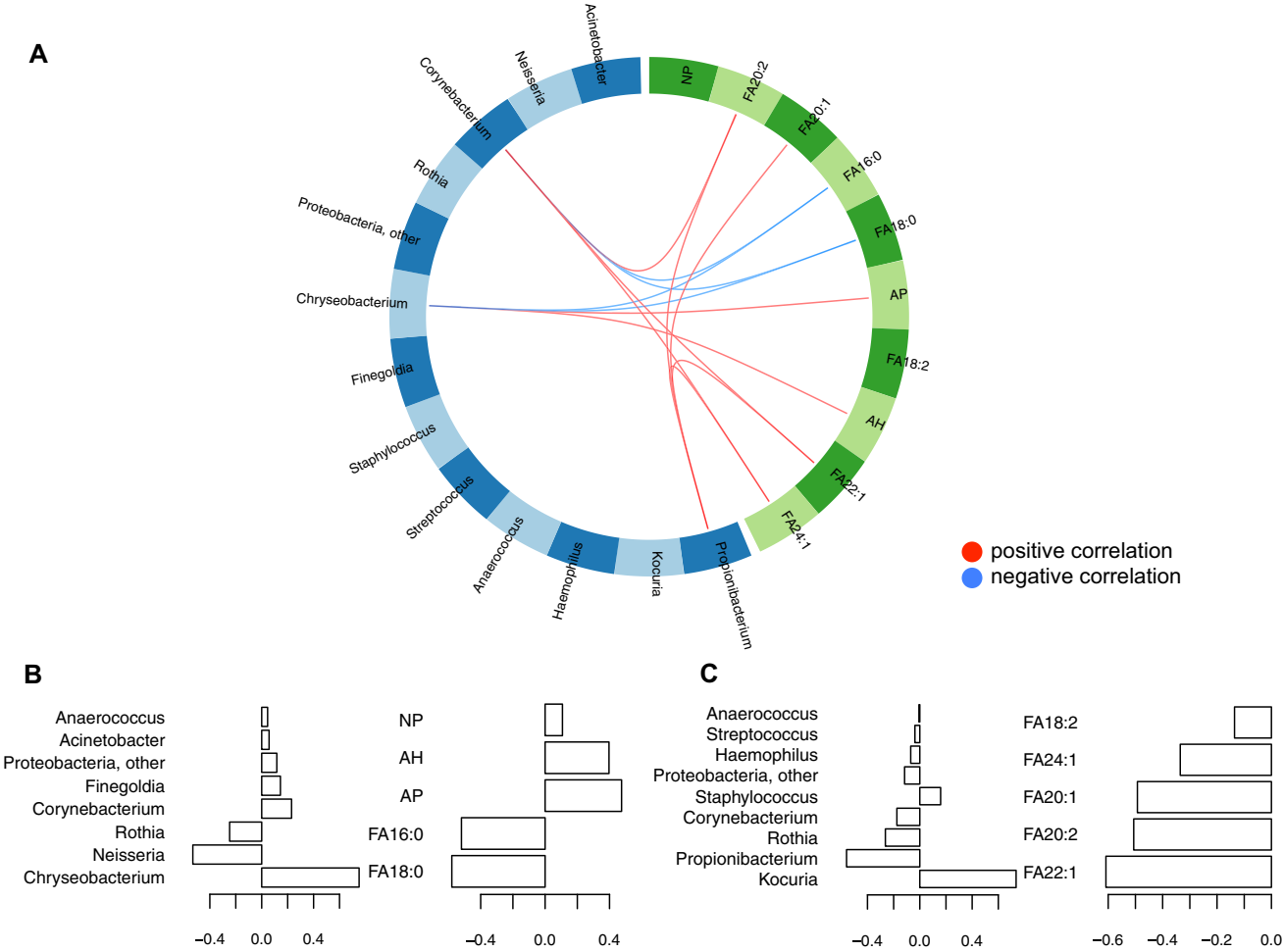


FIG E3. Effect of epidermal lipid composition on bacterial community composition. **A**, The Circos plot shows genera and epidermal lipids contributing to the overall correlation between bacterial abundance and levels of lipid species identified by using sparse canonical correlation analysis. The strongest pairwise positive (negative) correlations ($r > 0.6$) between selected genera and epidermal lipids are depicted. **B** and **C**, Coefficient weights of the selected genera and lipids on the first (Fig E3, **B**) and second (Fig E3, **C**) components of each omics level. *AH*, α -Hydroxy fatty acid/6-hydroxy-sphingosine base; *AP*, α -hydroxy fatty acid/phytosphingosine base; *NP*, nonhydroxy fatty acid/phytosphingosine base.

	Propioni- bacterium	Coryne- bacterium	Kocuria	Rothia	Staphylo- coccus	Strepto- coccus	Anaero- coccus	Finegoldia	Acineto- bacter	Haemo- philus	Neisseria	Chryseo- bacterium
Antecubital fossa												
AH	0.37	0.39	-0.14	-0.41	0.31	-0.27	0.45	0.43	0.18	-0.38	-0.43	0.33
AS	0.28	0.24	-0.20	-0.47	0.37	-0.45	0.47	0.47	-0.08	-0.50	-0.58	0.14
AP	0.36	0.28	-0.13	-0.67	0.17	-0.46	0.39	0.54	0.07	-0.56	-0.62	0.27
NP	0.46	0.32	-0.25	-0.66	0.27	-0.47	0.38	0.51	-0.09	-0.54	-0.65	0.12
FA16:0	-0.33	-0.27	-0.06	0.47	-0.13	0.45	-0.20	-0.44	-0.14	0.51	0.68	-0.53
FA18:0	-0.33	-0.30	-0.13	0.43	-0.14	0.46	-0.30	-0.54	-0.16	0.46	0.69	-0.50
FA18:2	0.65	0.46	-0.14	-0.08	0.06	0.16	0.00	0.06	0.05	0.12	-0.01	0.03
FA20:1	0.57	0.61	-0.20	-0.29	0.23	-0.33	0.42	0.46	0.00	-0.18	-0.56	0.10
FA20:2	0.52	0.41	-0.03	-0.45	0.36	-0.37	0.43	0.54	0.10	-0.44	-0.61	0.32
FA22:1	0.46	0.60	-0.31	-0.26	0.07	-0.31	0.30	0.41	-0.10	-0.09	-0.55	0.05
FA24:1	0.15	0.56	-0.49	-0.25	0.11	-0.36	0.30	0.35	-0.13	-0.11	-0.58	0.01
Forehead												
AH	-0.35	0.06	0.05	-0.30	-0.24	-0.18	-0.27	-0.12	0.46	-0.31	-0.13	0.51
AS	-0.63	-0.27	0.34	0.22	-0.41	0.02	-0.38	-0.34	0.13	-0.18	0.27	0.26
AP	-0.50	-0.24	-0.10	-0.04	-0.42	-0.19	-0.45	-0.35	0.20	-0.34	-0.13	0.48
NP	-0.43	-0.28	-0.38	0.12	-0.44	-0.24	-0.40	-0.46	-0.05	-0.31	-0.11	0.25
FA16:0	0.00	-0.10	0.11	0.39	-0.07	0.16	0.11	-0.25	-0.23	0.12	0.31	-0.49
FA18:0	0.01	-0.13	0.01	0.33	-0.15	0.07	0.02	-0.33	-0.24	0.02	0.24	-0.44
FA18:2	0.20	-0.09	-0.41	0.05	-0.19	-0.06	-0.16	-0.37	-0.30	-0.09	0.04	-0.33
FA20:1	-0.47	-0.26	-0.80	0.16	-0.71	-0.58	-0.61	-0.80	-0.40	-0.61	-0.28	0.06
FA20:2	0.17	0.11	-0.69	0.13	-0.01	0.13	-0.04	-0.09	-0.07	0.06	0.03	0.28
FA22:1	0.24	0.22	-0.62	0.36	0.10	0.38	0.13	-0.08	-0.15	0.27	0.32	0.04
FA24:1	0.16	0.22	-0.40	0.45	0.15	0.52	0.16	-0.02	-0.10	0.37	0.46	0.09
Volar forearm												
AH	0.21	0.32	-0.30	0.14	-0.13	0.40	0.43	0.16	0.46	0.40	-0.27	0.55
AS	0.19	0.24	-0.42	0.06	0.04	0.36	0.42	0.17	0.33	0.39	-0.33	0.37
AP	0.30	0.44	0.07	0.14	-0.52	0.32	0.33	-0.07	0.49	0.25	-0.27	0.66
NP	0.50	0.47	-0.23	0.04	-0.41	0.35	0.36	0.10	0.42	0.39	-0.29	0.62
FA16:0	-0.12	-0.25	-0.05	0.34	0.24	0.17	-0.12	-0.04	-0.04	0.16	0.59	-0.60
FA18:0	-0.19	-0.35	0.09	0.41	0.25	0.12	-0.26	-0.17	-0.13	0.02	0.48	-0.64
FA18:2	0.12	-0.28	0.00	0.45	-0.03	0.21	-0.37	-0.35	-0.16	0.05	0.16	-0.59
FA20:1	0.59	0.24	-0.11	0.53	-0.44	0.53	0.02	-0.12	0.19	0.39	0.05	-0.07
FA20:2	0.68	0.57	-0.03	0.32	-0.56	0.57	0.39	0.04	0.39	0.45	-0.27	0.34
FA22:1	0.48	0.23	-0.26	0.39	-0.43	0.40	0.02	0.01	0.16	0.29	-0.04	0.15
FA24:1	0.35	0.13	-0.22	0.22	-0.38	0.19	-0.09	0.02	0.06	0.08	-0.21	0.24

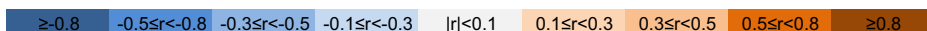


FIG E4. Correlation of bacterial genera with skin lipids at different body sites. *AH*, α -Hydroxy fatty acid/6-hydroxy-sphingosine base; *AS*, α -hydroxy fatty acid/sphingosine base; *AP*, α -hydroxy fatty acid/phytosphingosine base; *NP*, nonhydroxy fatty acid/phytosphingosine base. FFAs are depicted as FAXX:Y, with XX indicating chain length and Y indicating number of double bonds/degree of unsaturation.

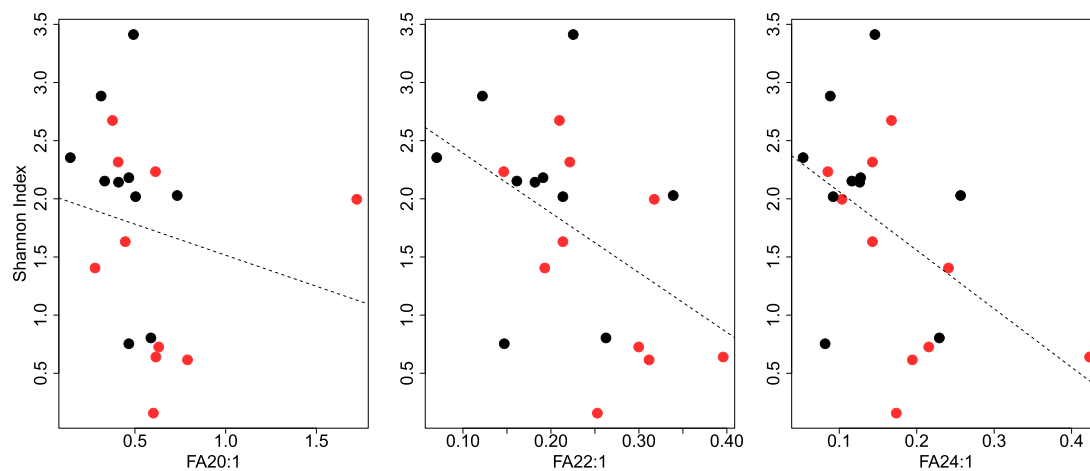


FIG E5. Reduced α -diversity is correlated with increased levels of unsaturated long-chain FFAs at the AD predilection site. Spearman correlation of α -diversity (Shannon index) with levels of 3 unsaturated long-chain FFAs at the Af are calculated. *Red dots* depict patients with AD, and *black dots* depict healthy subjects.

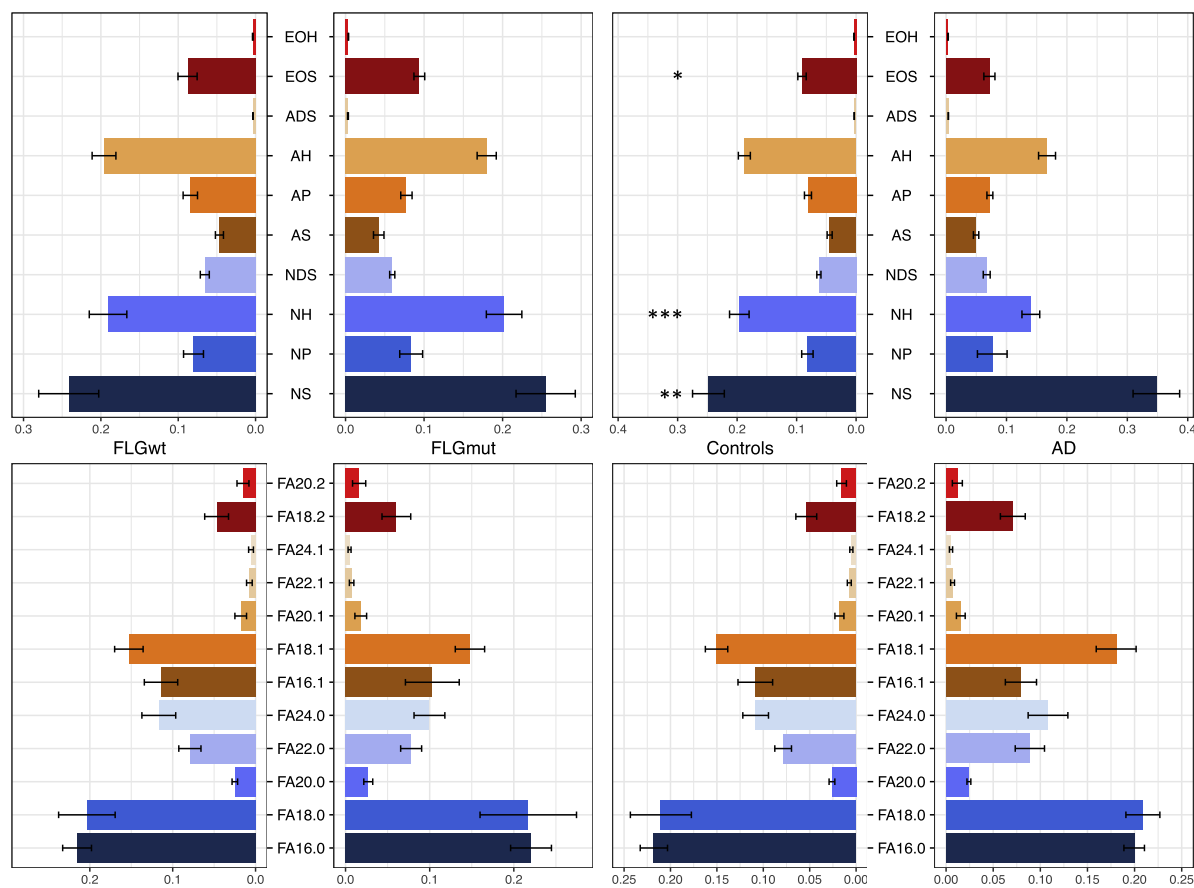


FIG E6. Association of the investigated ceramides and FFAs with FLG deficiency (*left*) and AD status (*right*). Asterisks indicate significance as follow: **P* < .05, ***P* < .005, and ****P* < .0005. For ceramides, the first letter depicts the fatty acid, and the remaining letters depict the respective sphingoid base. AX, α -Hydroxy fatty acid; DS, dihydrosphingosine base; EX, esterified ω -hydroxy fatty acid; NX, nonhydroxy fatty acid; XH, 6-hydroxy-sphingosine base; XP, phytosphingosine base; XS, sphingosine base. FFAs are depicted as FAXX:Y, with XX indicating chain length and Y indicating number of double bonds/degree of unsaturation.

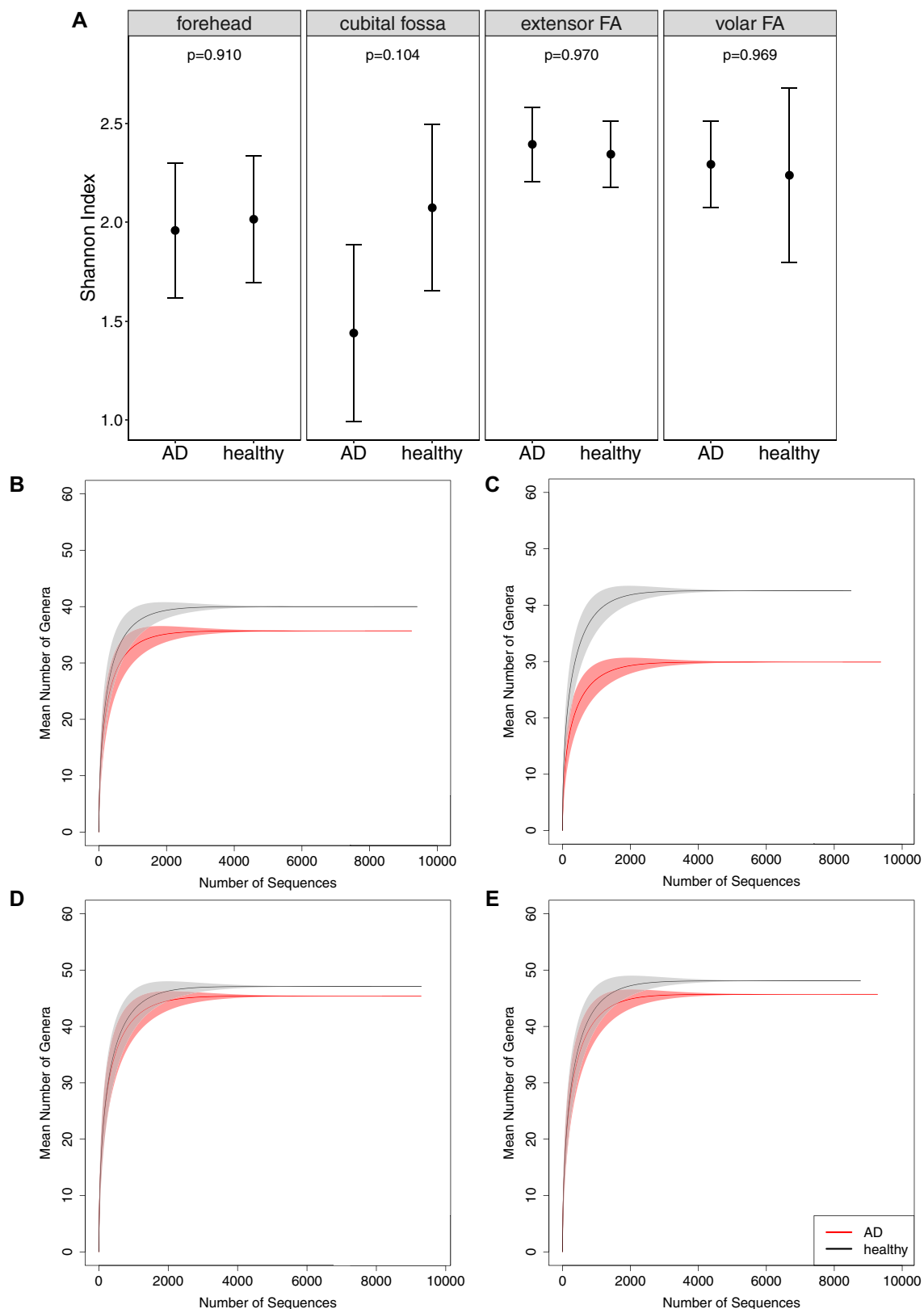


FIG E7. Reduced α -diversity and species richness in patients with AD at the AD predilection site. α -Diversity (Shannon index) and richness are calculated at the genus taxonomic level for patients with AD and healthy subjects. **A**, α -Diversity at all 4 sites. **B-E**, Species richness at the forehead (Fig E7, **B**), Af (Fig E7, **C**), Ef (Fig E7, **D**), and Vf (Fig E7, **E**). Rarefaction curves are depicted with 2 times the sampling error.

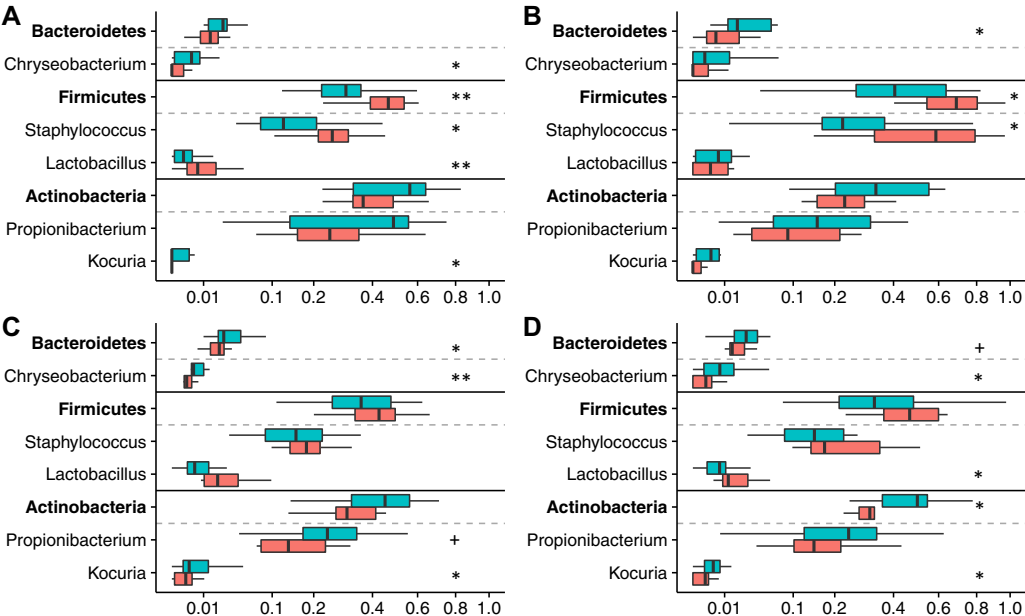


FIG E8. Effect of AD on the skin microbiome. Bacteroidetes, Firmicutes, and Actinobacteria genera showing differential abundance in patients with AD and healthy control subjects. For better visualization, abundance is depicted on the square root scale: **A**, forehead; **B**, Af; **C**, Vf; and **D**, Ef. Red bars depict patients with AD, and blue bars depict healthy control subjects. Asterisks indicate significance obtained from the Wilcoxon test as follows: + $P < .1$, * $P < .05$, and ** $P < .005$.

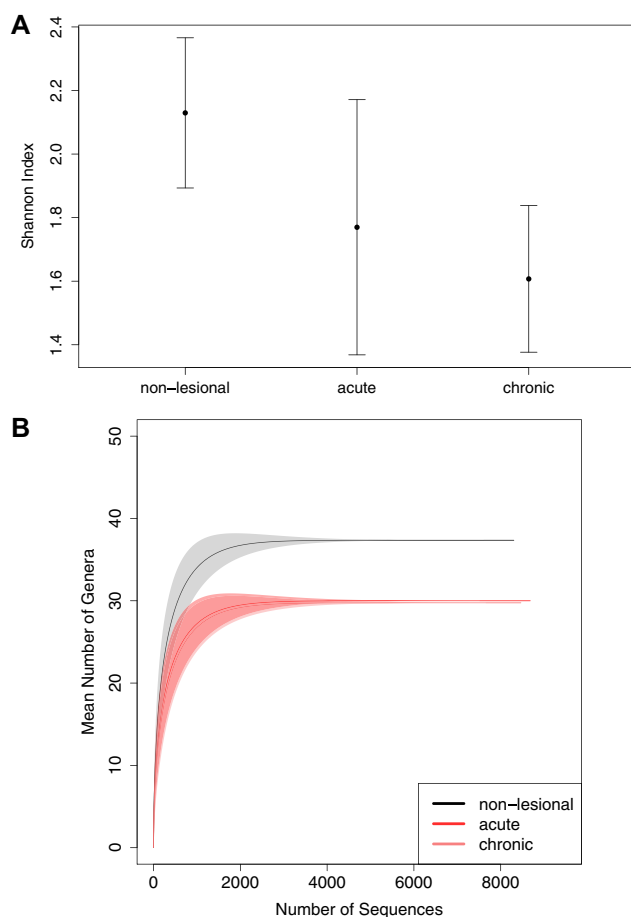


FIG E9. Reduced α -diversity and species richness in acute and chronic lesions. **A**, For patients with AD, α -diversity (Shannon index) is calculated at both nonlesional skin and acute and chronic lesions. *Non-lesional* reflects the mean diversity of the 4 body sites. **B**, Species richness at the genus taxonomic level for patients with AD at nonlesional skin, as well as at acute and chronic lesions. *Non-lesional* reflects the mean richness of the 4 body sites. Rarefaction curves are depicted with 2 times the sampling error.

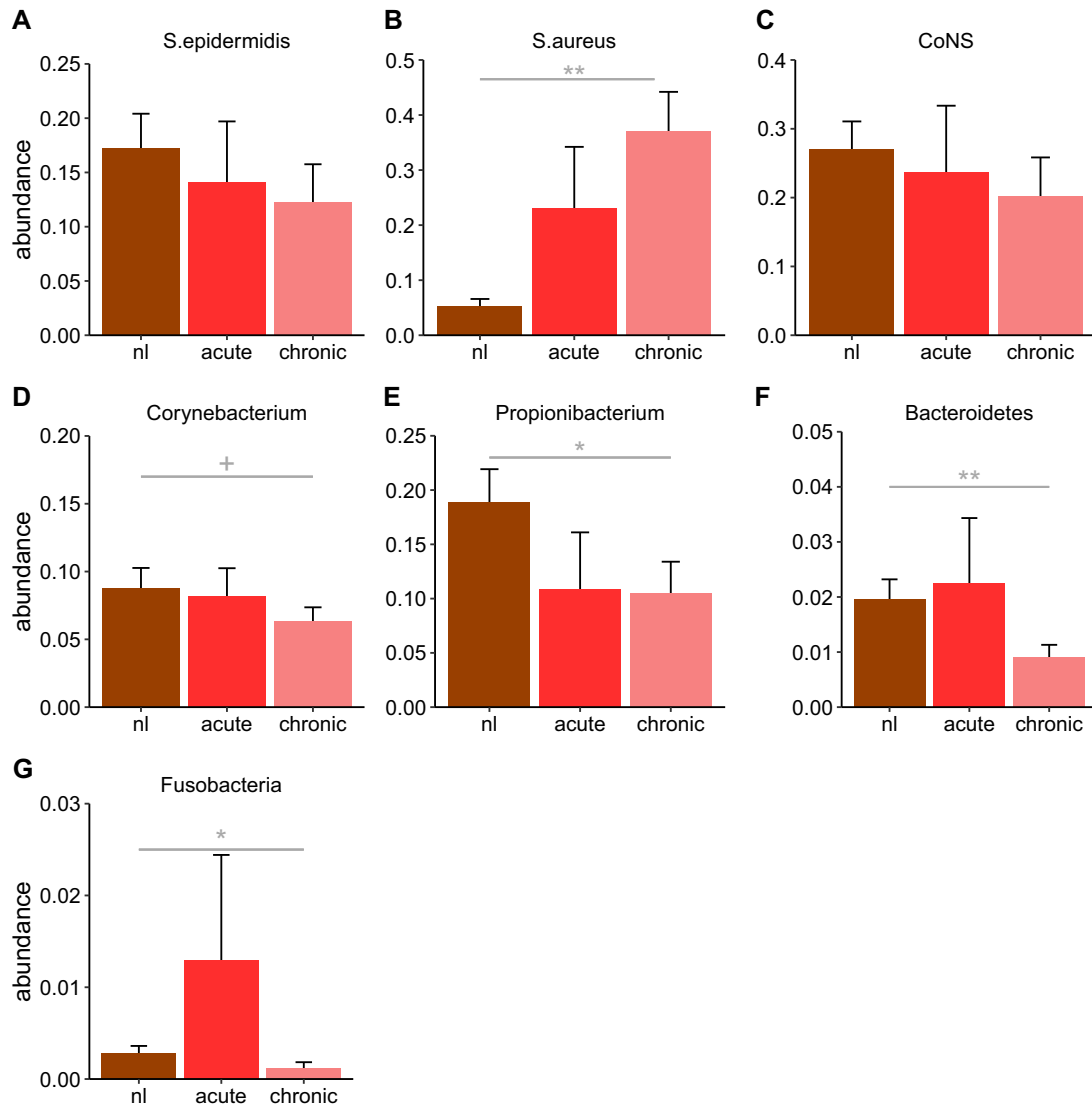


FIG E10. A-G, Differential bacterial abundance at nonlesional (nl) and acute and chronic lesional skin in patients with AD. Depicted is the mean $\pm$ SE abundance of the prevalent taxa with an abundance of at least 5%. Asterisks indicate significance obtained from the Wilcoxon test as follows: + $P < .1$, * $P < .05$, and ** $P < .005$.

TABLE E1. Proband characteristics

ID	FLG	Sex	Age (y)	SCORAD	Acute site	Chronic site
Co09	FLGwt	Male	29			
Co08	FLGwt	Male	41			
Co01	FLGwt	Female	27			
Co04	FLGwt	Female	27			
Co07	FLGwt	Female	54			
Co04	FLGmut	Male	36			
Co08	FLGmut	Female	39			
Co09	FLGmut	Female	25			
Co01	FLGmut	Female	32			
Co11	FLGmut	Female	44			
AD04	FLGwt	Male	26	29	Af (left)	wrist (left)
AD05	FLGwt	Male	48	33		Af (left)
AD10	FLGwt	Female	20	22		neck
AD03	FLGwt	Female	25	34	Vf (right)	Vf (left)
AD01	FLGwt	Female	46	31	Lower back	Lower back
AD09	FLGmut	Male	30	42	Chest	Af (left)
AD06	FLGmut	Male	45	46	Wrist (right)	Wrist (right)
AD08	FLGmut	Female	21	37	Upper arm (left)	Af (left)
AD02	FLGmut	Female	32	34		Wrist (right)
AD05	FLGmut	Female	51	29		Wrist (left)

TABLE E2. TrueMass stratum corneum lipid panel

FFAs			
Chain length	Saturated	Monounsaturated	Diunsaturated
16	16:0	16:1	
18	18:0	18:1	18:2
20	20:0	20:1	20:2
22	22:0	22:1	
24	24:0	24:1	

Ceramides			
Sphingoid base	Nonhydroxy	α -Hydroxy	Esterified ω -hydroxy
Ceramides	NS (65)	AS (37)	EOS (21)
Dihydroceramides	NDS (54)	ADS (7)	
6-Hydroxyceramides	NH (39)	AH (31)	EOH (12)
Phytoceramides	NP (41)	AP (31)	

Twelve FFA and 10 ceramide classes were measured. For the ceramide classes, a different number of lipid species is measured and depicted in parentheses. *ADS*, α -Hydroxy fatty acid/dihydrosphingosine base; *AH*, α -hydroxy fatty acid/6-hydroxy-sphingosine base; *AP*, α -hydroxy fatty acid/phytosphingosine base; *AS*, α -hydroxy fatty acid/sphingosine base; *NDS*, nonhydroxy fatty acid/dihydrosphingosine base; *NH*, nonhydroxy fatty acid/6-hydroxy-sphingosine base; *NP*, nonhydroxy fatty acid/phytosphingosine base; *NS*, nonhydroxy fatty acid/ sphingosine base.

TABLE E3. Mean bacterial abundance of Bacteroidetes, Firmicutes, and Actinobacteria genera in accounting for the abundance difference at the phylum level with regard to AD status

	Forehead			Af			Vf			Ef		
	Patients with AD	Healthy subjects	P value	Patients with AD	Healthy subjects	P value	Patients with AD	Healthy subjects	P value	Patients with AD	Healthy subjects	P value
Bacteroidetes	2.4% ± 1.0%	2.7% ± 0.5%	.9824	1.3% ± 0.5%	4.8% ± 2.1%	.0231	2.0% ± 0.3%	4.2% ± 1.5%	.0845	2.2% ± 0.3%	3.7% ± 0.8%	.0292
<i>Chryseobacterium</i>	0.1% ± 0.1%	0.6% ± 0.2%	.0210	0.3% ± 0.2%	1.2% ± 0.7%	.1381	0.3% ± 0.1%	1.3% ± 0.6%	.0106	0.3% ± 0.1%	1.1% ± 0.5%	.0054
Firmicutes	44.9% ± 4.1%	30.8% ± 4.3%	.0066	67.9% ± 5.9%	45.0% ± 8.2%	.0281	45.9% ± 4.9%	37.7% ± 8.1%	.2753	42.1% ± 4.5%	36.3% ± 5.1%	.3348
<i>Staphylococcus</i>	27.1% ± 4.7%	15.5% ± 4.1%	.0313	56.7% ± 8.9%	31.3% ± 8.4%	.0333	24.5% ± 5.0%	22.3% ± 8.6%	.7360	21.0% ± 3.9%	16.8% ± 3.3%	.3195
<i>S aureus</i>	2.8% ± 1.3%	0.3% ± 0.1%	5.4E-04	8.9% ± 3.9%	0.2% ± 0.1%	5.1E-17	6.5% ± 2.1%	0.4% ± 0.3%	3.3E-04	2.9% ± 0.7%	0.5% ± 0.4%	.0165
<i>S epidermidis</i>	17.8% ± 4.3%	10.2% ± 2.9%	.0782	32.4% ± 8.4%	13.8% ± 2.9%	.0049	10.6% ± 2.4%	14.6% ± 7.8%	.6165	8.0% ± 1.7%	8.0% ± 1.2%	.8875
<i>S hominis</i>	0.6% ± 0.2%	0.6% ± 0.2%	.9930	7.9% ± 4.4%	10.9% ± 5.7%	.7063	2.0% ± 0.7%	3.7% ± 0.9%	.2137	1.4% ± 0.3%	2.8% ± 0.6%	.0425
<i>Lactobacillus</i>	3.0% ± 1.9%	0.3% ± 0.2%	.0027	2.5% ± 2.0%	1.0% ± 0.4%	.1273	3.8% ± 2.0%	1.1% ± 0.4%	.0371	3.2% ± 0.9%	1.4% ± 0.7%	.1077
Actinobacteria	40.2% ± 4.3%	50.2% ± 6.3%	.4172	22.5% ± 3.6%	36.7% ± 6.2%	.2673	34.9% ± 4.6%	44.2% ± 6.5%	.3451	34.5% ± 5.1%	44.7% ± 5.7%	.2133
<i>Propionibacterium</i>	28.5% ± 5.8%	38.9% ± 7.9%	.3440	12.4% ± 3.3%	19.0% ± 5.0%	.6501	18.0% ± 3.8%	25.1% ± 5.9%	.3665	16.4% ± 3.0%	26.1% ± 5.0%	.0852
<i>Kocuria</i>	0.0% ± 0.0%	0.6% ± 0.4%	.0115	0.1% ± 0.1%	1.0% ± 0.5%	.1304	0.2% ± 0.1%	0.9% ± 0.4%	.0122	0.3% ± 0.1%	1.1% ± 0.5%	.0213

TABLE E4. Ratio of *Staphylococcus* species

	Patients with AD		Healthy subjects		RFC	95% CI	P value
	Median	Q1-Q3	Median	Q1-Q3			
<i>S aureus/S epidermidis</i>							
Forehead	0.056	0.029-0.151	0.034	0.003-0.076	5.9	0.8-42.2	.0937
Vf	0.613	0.241-0.960	0.003	0.001-0.016	85.6	22.9-320.2	6.9×10^{-6}
Ef	0.298	0.148-0.979	0.006	0.002-0.040	38.3	9.4-155.3	2.0×10^{-4}
Af	0.080	0.035-0.499	0.003	0.001-0.023	31.2	5.7-172.0	.0010
<i>S aureus/CoNS</i>							
Forehead	0.042	0.020-0.981	0.027	0.001-0.050	5.4	0.8-36.2	.1047
Vf	0.322	0.112-0.652	0.002	0.001-0.009	88.4	25.1-311.4	7.0×10^{-6}
Ef	0.142	0.092-0.173	0.003	0.001-0.021	30.8	8.0-117.8	4.0×10^{-4}
Af	0.077	0.029-0.142	0.002	0.001-0.008	38.3	6.5-225.7	8.0×10^{-4}

Ratios are obtained by calculating the proportion of species count A divided by species log(A/B). To avoid dividing by zero, we added 1 to each count. For hypothesis testing, ratios were log-transformed to obtain normality, and the *t* test was applied.

Q1, Lower quartile; Q3, upper quartile; RFC, ratio fold change.