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Angaben zur Veröffentlichung / Publication details:

Rauer, Luise, Matthias Reiger, Madhumita Bhattacharyya, P. M. Brunner, J. G. Krueger, E. Guttman Yassky, Claudia Traidl-Hoffmann, and Avidan Neumann. 2023. "Skin microbiome and its association with host cofactors in determining atopic dermatitis severity." *Journal of the European Academy of Dermatology and Venereology* 37 (4): 772–82. https://doi.org/10.1111/jdv.18776.

Received: 27 June 2022 | Accepted: 9 November 2022

DOI: 10.1111/jdv.18776

JEADV JOURNAL OF THE EUROPEAN ACADEMY OF DERMATOLOGY & VENEREOLOGY

ORIGINAL ARTICLE

Skin microbiome and its association with host cofactors in determining atopic dermatitis severity

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Funding information
Helmholtz Association, Germany: Impuls- und Vernetzungsfonds (IVF); Helmholtz Center Munich: Allergy-Projects

Abstract

Background: Atopic dermatitis (AD) is a heterogeneous, chronic inflammatory skin disease linked to skin microbiome dysbiosis with reduced bacterial diversity and elevated relative abundance of *Staphylococcus aureus* (*S. aureus*).

Objectives: We aimed to characterize the yet incompletely understood association between the skin microbiome and patients' demographic and clinical cofactors in relation to AD severity.

Methods: The skin microbiome in 48 adult moderate-to-severe AD patients was investigated using next-generation deep sequencing (16S rRNA gene, V1–V3 region) followed by denoising (DADA2) to obtain amplicon sequence variant (ASV) composition.

Results: In lesional skin, AD severity was associated with *S. aureus* relative abundance ($r_S = 0.53$, $p < 0.001$) and slightly better with the microbiome diversity measure Evenness ($r_S = -0.58$, $p < 0.001$), but not with Richness. Multiple regression confirmed the association of AD severity with microbiome diversity, including Shannon (in lesional skin, $p < 0.001$), Evenness (in non-lesional skin, $p = 0.015$) or *S. aureus* relative abundance ($p < 0.012$), and with patient's IgE levels ($p < 0.001$), race ($p < 0.032$), age ($p < 0.034$) and sex ($p = 0.012$). The lesional model explained 62% of the variation in AD severity, and the non-lesional model 50% of the variation.

Conclusions: Our results specify the frequently reported "reduced diversity" of the AD-related skin microbiome to reduced Evenness, which was in turn mainly driven by *S. aureus* relative abundance, rather than to a reduced microbiome Richness. Finding associations between AD severity, the skin microbiome and patient's cofactors is a key aspect in developing new personalized AD treatments, particularly those targeting the AD microbiome.

INTRODUCTION

Atopic dermatitis (AD) is a chronic, inflammatory skin disease with a severely reduced quality of life, affecting approximately 7% of adults in Western countries and up to 25% of children.[1–5] Along with the presence of heterogenous phenotypes and endotypes,[6–8] the aetiology of this complex disease is a matter of ongoing debate.[9] AD pathogenesis has been linked to multiple genetic and environmental risk factors, such as an impaired skin barrier function by mutations

Linked article : F. Rieder and P.K. Mukherjee. J Eur Acad Dermatol Venereol 2023; 37:649-650. https://doi.org/10.1111/jdv.18776.

in the filaggrin gene, as well as immune dysregulation in the Th2 and Th22 inflammatory pathways.[2,9] In addition, advances in microbial culture-independent sequencing techniques have revealed a *Staphylococcus aureus*-related skin microbiome dysbiosis in AD.[10] Specifically, the AD skin microbiome is characterized by decreased microbial diversity and compositional changes in comparison to healthy subjects.[10,11] Most notably, *S. aureus* is predominantly found in the lesional skin of AD patients and is associated with disease severity.[12–14] Despite the early discovery of this association[15] and the intensive research focused on *S. aureus*, evidence of a causal role of this opportunistic pathogen in AD is still lacking.[10] The unaffected (i.e. non-lesional) skin of AD patients is in an intermediate state between healthy and AD lesional skin in terms of physiological properties, *S. aureus* colonization and microbial diversity.[12,13,16,17]

The low microbial diversity observed in AD can be affected by the two distinct sub-components of alpha diversity: richness, which measures the number of different taxa present, and evenness, which describes how equally or skewed the taxa relative abundances are distributed.[18] However, microbiome research in AD rarely used these distinct measures of alpha diversity, but rather reported "mixed" measures, such as Inverse Simpson's or Shannon's diversity index, which are affected by both of the distinct components richness and evenness.[12,14,19–23] Thus, it remains to be investigated whether the decreased microbial diversity described in AD is attributed to a depletion of taxa (lower richness) or to a more imbalanced distribution of taxa (lower evenness) in the skin microbiome, and which of the diversity indices is best associated with AD severity.

In addition, it is still not clear to what extent the skin microbiome dysbiosis and *S. aureus* contribute to the development and progression of AD.[11,24,25] Moreover, the influence of host-associated factors like sex, age and race – which have been shown to shape the microbiota in healthy individuals[26,27] – on the AD patients' microbiome and disease severity is not yet well investigated. Advancing knowledge about the AD-associated microbiome and its relation to demographic cofactors may improve our understanding of AD pathogenesis and lead to new targeted therapies, biomarkers and disease prediction models.[10,26] This study investigates the link between skin microbiome in AD and disease severity, characterizing the composition and diversity of the skin microbiome in 48 moderate-to-severe AD patients[28,29] in relation to AD severity and demographic variables.

MATERIALS AND METHODS

Study design

Complete methods can be found in the Appendix S1. Briefly, we investigated baseline demographic and microbiome data from a cohort of 60 moderate-to-severe AD patients recruited in New York, USA (clinicaltrials.gov, no. NCT01941537).[28,29] Inclusion criteria included a scoring atopic dermatitis (SCORAD) score ≥30, and patients were washed out from previous use of systemic and topical treatments. All participants provided written informed consent before inclusion. Of this cohort, clinical outcomes and transcriptomic changes during the drug treatment have already been reported.[28,29] Here, we are investigating for the first time the microbiome data at baseline, which was available for a subset of $n = 49$ patients prior to enrolment in the clinical trial.

Microbiome sampling and sequencing

Skin microbiome was sampled by swabbing lesional and adjacent non-lesional skin (Table S1 in Appendix S1). Cells were mechanically lysed, and microbial DNA was extracted following the QIAamp UCP Pathogen Mini Kit (QIAGEN) protocol. During PCR amplification, the hypervariable regions V1–V3 of the 16S rRNA gene were amplified. Samples were equimolarly pooled and analysed together with positive and negative controls via multiplexed bidirectional sequencing (2 × 300 base pairs) using a MiSeq® system (Illumina). Sequences were denoised using DADA2[30] through QIIME2,[31] and 16S sequences of all amplicon sequence variants (ASVs) were annotated using the AnnotIEM software[32] and the RDP database.[33] ASVs that represent singletons, contaminants, or eukaryotes were removed from downstream analysis, and samples with <2000 reads ($n = 4$ samples) or of moist skin type ($n = 2$ samples) were excluded, leading to a total of $n = 48$ patients for analysis.

Statistical analysis

For statistical correlations with AD severity, we used objective SCORAD (oSCORAD), which considers only extent and intensity of AD. oSCORAD excludes the subjective factors sleep loss and pruritus of the SCORAD, which may be influenced by social and cultural background.[34,35]

All statistical analyses were performed using the statistical software package R.[36] Non-parametric statistical tests were chosen for all analyses, namely Spearman's rank correlation coefficient (correlation coefficient rho indicated by r_S), Mann–Whitney U test, Kruskal–Wallis test, Wilcoxon signed-rank test for paired data, and Fisher's exact test for categorical variables. Raw p-values ≤0.05 from two-sided tests were considered statistically significant. Intercorrelations between species were estimated using Spearman's rank correlation coefficient on CLR-transformed abundances.

Several alpha diversity indices were used to assess different aspects of bacterial diversity within samples: number of ASVs present ('Richness') and Normalized Shannon Entropy ('Evenness', also known as Pilou's J) as the two distinct components of alpha diversity; Shannon diversity index ('Shannon') and Inverse Simpson diversity index ('Inverse Simpson') as mixed measures of alpha diversity, incorporating both of the distinct alpha diversity components richness and evenness.[18] Venn diagram analysis of shared

taxa between groups was performed on all species present in at least 10% of lesional or non-lesional samples.

Beta diversity between samples was estimated using Bray-Curtis dissimilarities and visualized by non-metric multidimensional scaling (nMDS). Statistical significance between groups was assessed using a permutational analysis of variance (PERMANOVA) test. For stacked bar plots showing taxonomic distributions, ASVs with identical taxonomy were summarized into species.

Associations between main variables and oSCORAD were confirmed by multiple regression with backward elimination.

RESULTS

Study population

A total of 89 skin swabs from 48 moderate-to-severe AD patients were analysed, including 43 lesional and 46 non-lesional samples, with paired microbiome data available for 41 patients. Among patient characteristics (Table 1), only total serum IgE levels ($p = 0.0047$) were significantly different between moderate ($15 < \text{oSCORAD} < 40$) and severe ($\text{oSCORAD} \geq 40$) AD,[37] although IgE groups based on intrinsic (IgE < 200 kU/L) versus extrinsic (IgE ≥ 200 kU/L) AD were similar between moderate and severe AD.

Microbiome characteristics

The AD patients' skin microbiome (Figure 1; Figure S1) was dominated by *S. aureus*, which was detected in 79% of

TABLE 1 Patient characteristics and main skin microbiome species of the study population

	All ($n = 48$)	Moderate AD[a] ($n = 17$)	Severe AD[a] ($n = 31$)	p-Value
Patient characteristic				
Objective SCORAD, mean (SD)	44 (11.1)	34.2 (3.2)	49.4 (10.1)	–
Objective SCORAD, range	27.5–71.5	27.5–39	40.4–71.5	
Age, mean (SD)	41.5 (15)	38.5 (14)	43.1 (15.6)	0.24
BMI[b], mean (SD)	27.3 (6.2)	27.6 (5.8)	27.2 (6.5)	0.58
Sex, *n*				
Female	22 (46%)	9 (53%)	13 (42%)	0.55
Male	26 (54%)	8 (47%)	18 (58%)	
Race, *n*				
Asian-American	12 (25%)	2 (12%)	10 (32%)	0.28
African-American	21 (44%)	8 (47%)	13 (42%)	
Caucasian	15 (31%)	7 (41%)	8 (26%)	
IgE group, *n*				
Intrinsic (IgE < 200 kU/L)	10 (21%)	6 (35%)	4 (13%)	0.13
Extrinsic (IgE ≥ 200 kU/L)	38 (79%)	11 (65%)	27 (87%)	
Total serum IgE in kU/L, mean (SD)	5051 (7293)	1807 (2563)	6830 (8401)	0.0047
Microbiome characteristic				
S. aureus median rel. abundance				
Lesional samples ($n = 43$)	14.4%	1.4%	35.6%	0.012
Non-lesional samples ($n = 46$)	7.3%	0.3%	11.6%	0.05
S. epidermidis median rel. abundance				
Lesional samples ($n = 43$)	5.1%	9.5%	4.6%	0.51
Non-lesional samples ($n = 46$)	6.1%	3.6%	7.8%	0.85
C. acnes median rel. abundance				
Lesional samples ($n = 43$)	2.4%	3.9%	0.7%	0.038
Non-lesional samples ($n = 46$)	6.7%	6.4%	7.1%	0.89

Note: p-Values were obtained by the Mann–Whitney U test for continuous variables and Fisher's exact test for categorical variables.

Abbreviations: rel. abundance, relative abundance; SD, standard deviation.

[a]AD severity was defined by objective SCORAD (moderate: $15 \leq \text{oSCORAD} \leq 40$, severe: $\text{oSCORAD} > 40$).

[b]BMI values missing for two patients.

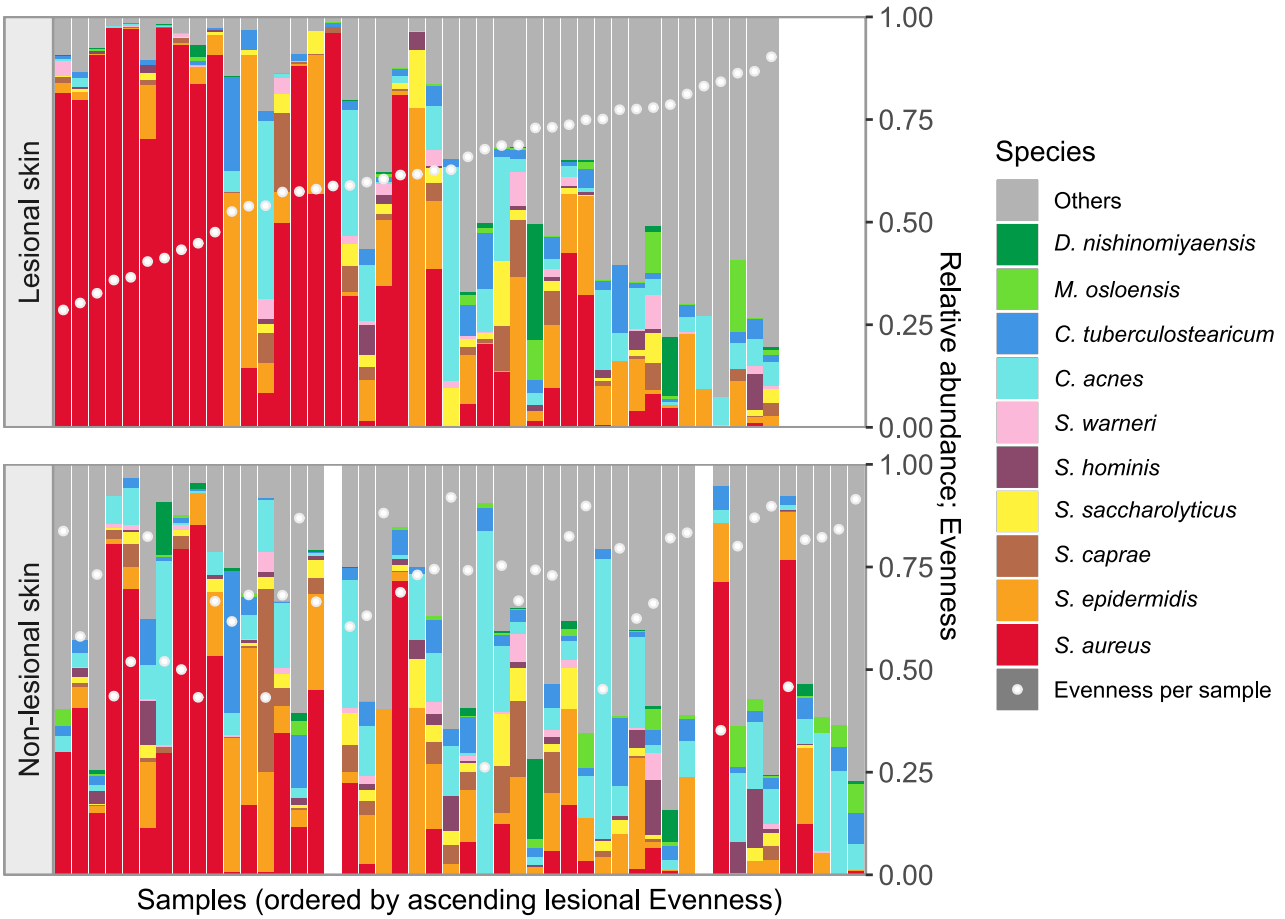

FIGURE 1 Microbiome sample composition in lesional and non-lesional skin and its association with microbiome Evenness (alpha diversity). The AD patients' skin microbiome is dominated by *S. aureus*, particularly in lesional skin samples. Low Evenness is mainly associated with high *S. aureus* relative abundance (except for few subjects with high relative abundance of *S. epidermidis* or *C. acnes*), while high-Evenness samples have almost no *S. aureus*, both in lesional and non-lesional skin. Microbiome composition is shown for the 10 most abundant species. Samples in lesion and non-lesion are ordered paired by subjects, according to ascending Evenness in lesion.

samples and was the most abundant species in 49% of lesional and 28% of non-lesional samples, followed by *Staphylococcus epidermidis* and *Cutibacterium acnes*. Significant differences between lesional and non-lesional skin were observed for relative abundances of *S. aureus* ($p < 0.001$) and *C. acnes* ($p = 0.0017$). Among the top 10 species, we found moderately strong negative correlations between *S. aureus* and *C. acnes* in lesional skin ($r_S = -0.43$, $p = 0.005$) and between *S. epidermidis* and *M. osloensis* in non-lesional skin ($r_S = -0.43$, $p = 0.003$). Positive associations in both lesional and non-lesional skin were found between the Staphylococci *S. caprae*, *S. saccharolyticus*, and *S. warneri* ($r_S \geq 0.50$, $p < 0.001$) (Figure S2).

The different skin locations sampled in this study (Table S1 in Appendix S1) were combined for analysis because we did not observe significant differences in the global microbiome composition between skin types (Figure S3a,b).

Microbiome alpha diversity

Regarding within-sample alpha diversity, only Evenness was significantly lower in lesion as compared to non-lesion ($p = 0.006$). Lower diversity in lesion compared to non-lesion was also observed as a trend for the Shannon and Inverse Simpson diversity indices (which take the distinct alpha diversity component evenness into account), but not for Richness (Figure 2a). Assessing the relation between lesional alpha diversity and AD severity, oSCORAD correlated best with Evenness ($r_S = -0.58$, $p < 0.001$), independent of skin sampling location (Figure S4a). oSCORAD was also associated with the mixed alpha diversity measures Shannon and Inverse Simpson ($r_S < -0.52$, $p < 0.001$), but not with Richness ($r_S = -0.28$, $p = 0.07$) (Figure 2b). Thus, high AD severity is associated with low Evenness, indicating an imbalanced distribution of microbiome taxa in lesional skin. In non-lesional skin, no significant correlation was found between alpha diversity and AD severity.

To verify that there is no association between Richness and AD skin status, the degree of overlap was investigated using Venn diagrams for all species present in at least 10% of

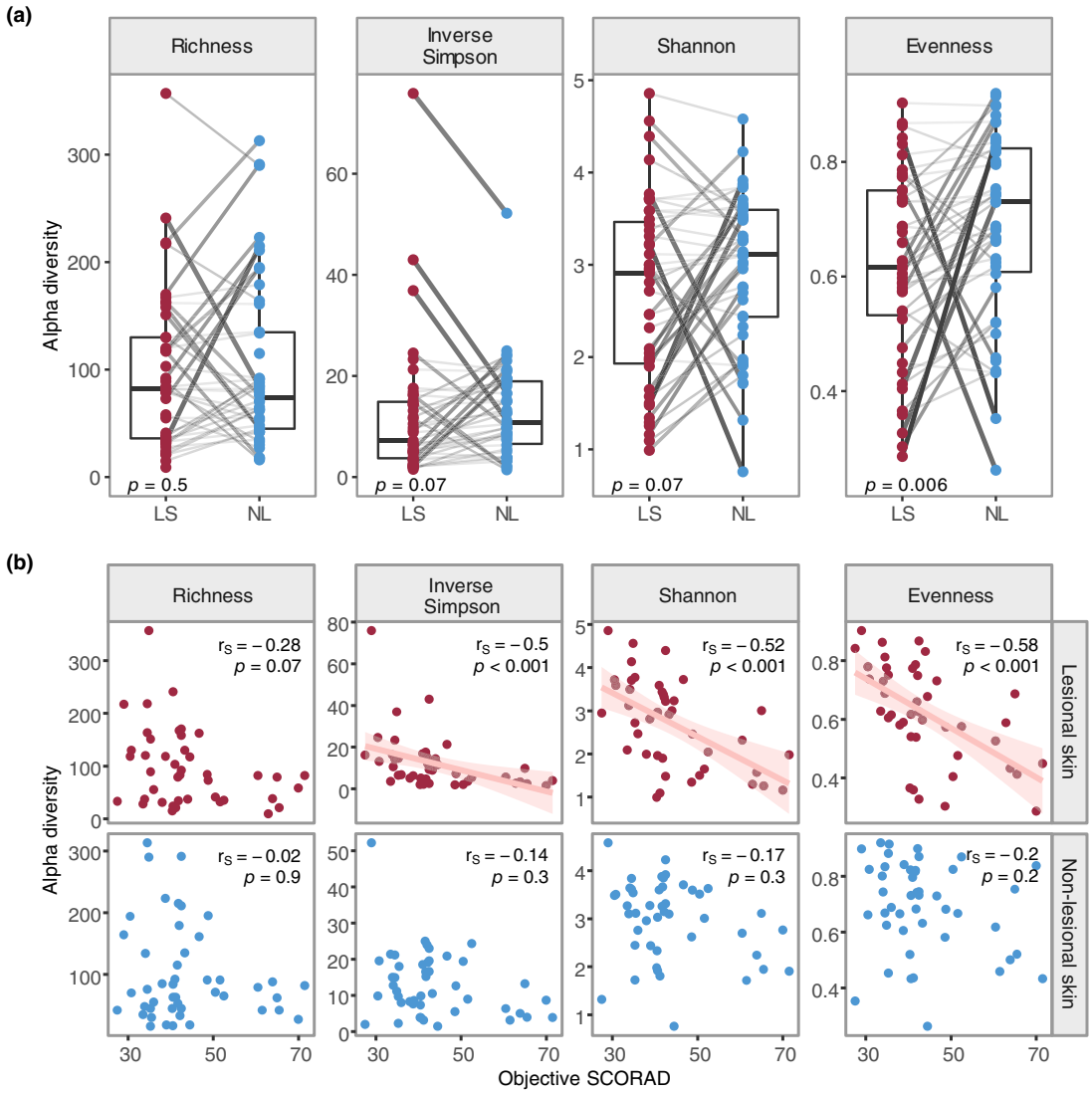

FIGURE 2 Alpha diversity in lesional and non-lesional skin and association with disease severity. Differences in diversity indices Richness, Inverse Simpson, Shannon and Evenness between lesional and non-lesional skin (a) and in correlation with AD severity in lesional and non-lesional skin (b) demonstrate a strong association of Evenness with AD skin status and severity. *p*-Values were obtained by a Wilcoxon signed-rank test on all paired samples (a) and by Spearman correlations in all samples (b). Boxes denote the median and interquartile range (IQR), whiskers represent values up to 1.5 times the IQR. Dots indicate individual samples, grey lines connect paired samples and line thickness represents the absolute slope. LS, lesional skin; NL, non-lesional skin; r_S, Spearman's rank correlation coefficient rho

lesional or non-lesional samples. We found that lesional and non-lesional samples shared all 183 species (Figure S5a), and that high AD severity was not characterized by a reduced number of species in either skin status (Figure S5b), supporting that Richness is not associated with skin status or AD severity in our data.

As Evenness correlated best with AD severity, we tested its interaction with the relative abundances of the most abundant species to identify the taxon that contributes the most to the observed reduced Evenness. Low-level Evenness was mostly characterized by high relative abundances of *S. aureus*, and high-level Evenness was characterized by relatively low *S. aureus* abundance (Figure 1). However, it can be seen that the dominance of other species like *S. epidermidis* or *C. acnes* can also lead to low Evenness values in some samples. Nevertheless, among the three most abundant species, relative abundance of *S. aureus* had the strongest correlation with Evenness ($r_S = -0.76$, $p < 0.001$) (Figure S6). Similarly, these findings apply to non-lesional skin sites as well, although the associations were generally weaker (Figure 1; Figure S6).

To further investigate the extent to which AD severity is correlated with *S. aureus* versus Evenness, alpha diversity indices were recalculated excluding *S. aureus*. Without *S. aureus* in the calculation of alpha diversity, the correlation of Evenness with oSCORAD became non-significant (Figure S7). Furthermore, excluding *S. aureus* removed all associations of alpha diversity with lesional versus non-lesional skin sites (Figure S7), identifying *S. aureus* as the

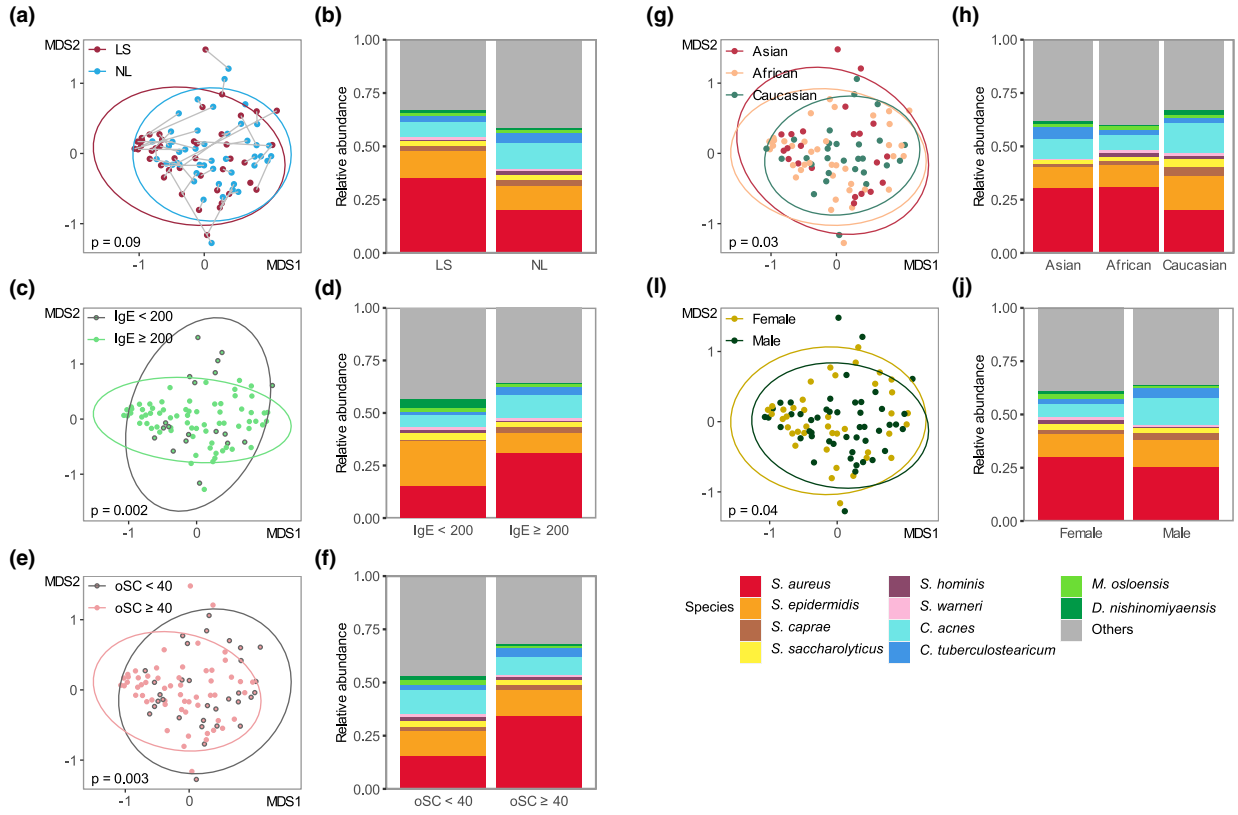

FIGURE 3 Univariable associations of beta diversity and microbiome composition with skin status and demographic factors. Beta diversity analysis revealed significant differences in the global microbiome for intrinsic versus extrinsic IgE levels (c, d), moderate versus severe AD (e, f), race (g, h), and sex (i, j) in lesional and non-lesional skin together, but not for lesional versus non-lesional skin (a, b). Beta diversity is visualized by nMDS on Bray-Curtis dissimilarities (a, c, e, g, i), *p*-values are derived from PERMANOVA tests with 1000 permutations and ellipses denote 95% confidence intervals around cluster centroids. Bar plots show mean microbiome composition of the 10 most abundant species (b, d, f, h, j). LS, lesional skin; NL, non-lesional skin; oSC, objective scoring atopic dermatitis

species mainly inducing the associations of alpha diversity with AD severity and AD-affected skin sites.

Microbiome beta diversity

Despite the differences in the relative abundances of *S. aureus* and *C. acnes* between lesional and non-lesional skin samples, the global microbiome composition (beta diversity) was not significantly different between the two skin sites ($p = 0.09$) (Figure 3a,b). Assessing associations between beta diversity and patient cofactors, significant results were found for intrinsic versus extrinsic IgE levels ($p = 0.002$), moderate versus severe AD ($p = 0.003$), race ($p = 0.03$), and for sex ($p = 0.04$) (Figure 3c–j), whereas no significant variations in the microbiome composition were found between age or BMI groups ($p = 0.2$) (Figure S3c–f).

Association between AD severity, microbiome and cofactors

In order to better understand the relation of AD severity with the microbiome and additional cofactors, univariate and multifactorial linear regression analysis was performed predicting oSCORAD from demographic and microbiome cofactors. Independent variables included the main cofactors age, BMI, sex, race, and IgE levels, as well as the relative abundances of the three major species, with or without alpha diversity indices. Analysis was performed separately in lesional and non-lesional skin samples in order to comply with the assumption of independent observations.

For lesional skin (Table 2), when alpha diversity measures were included in the analysis, the model revealed that only race ($p < 0.012$), IgE levels ($p < 0.001$), Shannon diversity ($p < 0.001$), and age ($p = 0.026$) were significantly associated with AD severity, explaining a substantial variation in AD severity ($R^2 = 62\%$, $p < 0.001$). Interestingly, the final model contains Shannon and not Evenness as a microbiome diversity measure, despite the superiority of Evenness in previous univariate analyses (Figure 2). When alpha diversity measures were a priori excluded from the analysis, the model performed nearly as well ($R^2 = 56\%$, $p < 0.001$) and confirmed the relation of AD severity with race ($p < 0.032$), IgE levels ($p < 0.001$), and age ($p = 0.034$), but also showed an association with *S. aureus* relative abundance ($p = 0.012$) instead of Shannon.

TABLE 2 Univariate and multiple regression explaining oSCORAD by demographic and microbiome characteristics in lesional skin

LS (n = 43)	Univariate analysis	Multiple regression with alpha diversity	Multiple regression without alpha diversity
Variable	**Estimate (p-Value)**	**Estimate (p-Value)**	**Estimate (p-Value)**
Race (Reference: Asian-American)			
African-American	−9.4 (0.028)	−9.8 (<0.001)	−10.1 (0.001)
Caucasian	−8.8 (0.053)	−7.6 (0.012)	−6.9 (0.032)
Total serum IgE [log10]	7.2 (<0.001)	6.3 (<0.001)	6.1 (<0.001)
S. aureus relative abundance	15.9 (<0.001)	n.s.	9.4 (0.012)
Shannon	−6.1 (<0.001)	−4.2 (<0.001)	a priori excluded
Age	n.s.	0.17 (0.026)	0.18 (0.034)
		adj. R^2 = 62% (<0.001)	**adj. R^2 = 56% (<0.001)**

Note: Other variables tested (BMI, sex, *C. acnes* relative abundance, *S. epidermidis* relative abundance, Evenness, Inverse Simpson, Richness) were not significant in the multiple regression models.

Abbreviation: n.s., not significant.

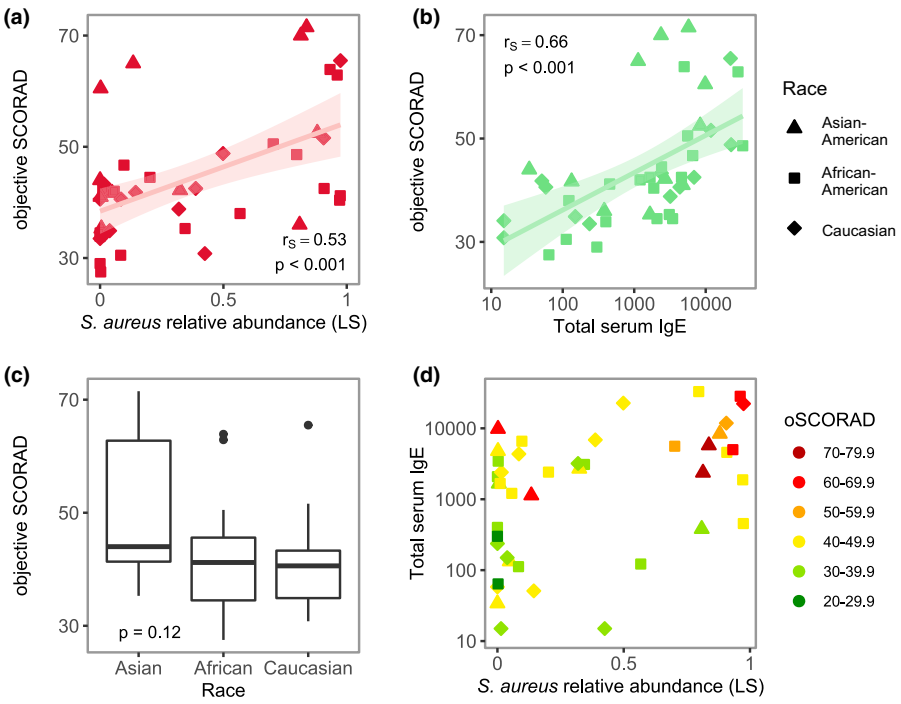

FIGURE 4 Association of AD severity with *S. aureus* relative abundance, IgE levels and race in lesional skin. Both lesional *S. aureus* relative abundance (a) and IgE levels (b) significantly correlate with AD severity as measured by oSCORAD. The differences in AD severity by race are not significant (c), although there is a trend for higher severity in patients of Asian-American race. Combining all three cofactors (d) shows that both *S. aureus* relative abundance and IgE levels contribute independently to severe AD, except for two Asian-American outliers with low *S. aureus* relative abundance and high AD severity. LS, lesional skin; r_S, Spearman's rank correlation coefficient rho

Investigating these associations in more detail, AD severity was significantly positively correlated with *S. aureus* relative abundance (r_S = 0.53, p < 0.001) and IgE levels (r_S = 0.66, p < 0.001) (Figure 4a,b). However, not all patients followed this trend. Notably, a few datapoints in the upper halves of the panels are characterized by particularly high oSCORAD, medium-to-high IgE levels, a wide range of *S. aureus* relative abundances and Asian-American race. Indeed, Asian-American participants tended to have slightly higher AD severity than African-American or Caucasian participants (p = 0.12, Figure 4c). Analysing the combined effects of both *S. aureus* relative abundance and IgE levels on oSCORAD (Figure 4d) – as determined before by multiple regression – visualizes the statistically independent contribution of both cofactors to AD severity. Nevertheless, IgE levels were significantly positively correlated with *S. aureus* relative abundance in lesional samples (r_S = 0.48, p = 0.001), but of note, many patients displayed low *S. aureus* relative abundance and high IgE levels, while barely any patient presented high *S. aureus* relative abundance and low IgE levels.

Investigating the joint effects of *S. aureus*, IgE, and race on AD severity, it seems that AD patients with particularly high oSCORAD tend to have both high IgE levels and high relative abundance of *S. aureus*, except for two Asian-American patients with low relative *S. aureus* abundance but high AD severity. Since different skin locations might influence *S. aureus* abundance in the skin, we have verified that the influence of sampling location did not affect this result (Figure S4b).

For non-lesional skin, the derived models from multiple regression were similar (R^2 = 50%, $p < 0.001$) (Table S2 in Appendix S1) and substantiated the strong influence of IgE levels ($p < 0.001$) and race ($p < 0.027$). In contrast to the lesional models, the best non-lesional model with alpha diversity includes Evenness (p = 0.015) and sex (p = 0.012) instead of Shannon and age as in the lesional skin model. When alpha diversity measures were excluded, *S. aureus* relative abundance was significantly associated with oSCORAD (p = 0.0046) also in non-lesional skin. The individual and combined effects of *S. aureus* relative abundance, IgE levels, and race on AD severity in non-lesional samples are visualized in Figure S8, revealing weaker associations between *S. aureus* and oSCORAD in non-lesion compared to lesional skin.

DISCUSSION

In the present study, the characterization of the skin microbiome in patients with moderate-to-severe AD revealed a profound association of the microbial composition and diversity with AD severity. In lesional skin, oSCORAD was negatively correlated with microbiome diversity, and specifically with Evenness rather than Richness, and positively correlated with *S. aureus* relative abundance. Moreover, both the microbiome and AD severity were strongly associated with IgE levels and race. Our multiple regression revealed that the association between the microbiome and AD severity is also dependent on patient demographic covariates like age, sex, IgE levels and race.

Regarding alpha diversity, only Evenness distinguished significantly between lesional and non-lesional skin, whereas neither Richness nor Shannon or Inverse Simpson were different between the two skin sites. Moreover, oSCORAD was correlated with the Shannon, Inverse Simpson and Evenness measures of the lesional skin microbiome diversity; however, Evenness correlated best with oSCORAD and there was no association observed between Richness and AD severity. In the multiple regression, AD severity was significantly associated with Shannon or Evenness, but never with Richness. These findings jointly suggest that the positive associations of AD severity with the Shannon and Inverse Simpson diversity indices – which are affected by the two distinct components of alpha diversity richness and evenness – are solely attributed to differences in species evenness and not to species richness. Thus, severe AD is associated with an imbalanced skin microbiome distribution (low Evenness) rather than a depletion in the number of taxa present (low Richness).

While the positive association of AD severity with mixed microbiome diversity measures like the Shannon index has been observed in previous studies as well,[12,14,19,20,38] Evenness as a distinct measure has rarely been investigated in AD.[39] Contrasting research that described a change of Richness in AD severity status has been reported in children.[12,20] In line with substantial alterations in the microbiome by age in both healthy[40,41] and AD-affected subjects,[23] the different aspects of microbial alpha diversity in AD skin might also depend on age. Our findings contribute to better understanding the frequently reported ambiguous "low diversity" in the AD skin microbiome as an "imbalanced" AD skin microbiome instead of a "depleted" one. Since Evenness correlated best with oSCORAD in our univariate analyses, we propose to add Evenness to the frequently reported alpha diversity measures, and to validate its association with AD severity.

AD severity was also strongly correlated to *S. aureus* relative abundances in our data, validating once more the positive correlation between *S. aureus* and AD severity,[12,14,20,21,39,42,43] here in a cohort of adult AD patients with moderate-to-severe disease. The association of AD severity with Evenness and *S. aureus* relative abundance, as well as the strong negative correlation of *S. aureus* relative abundance with Evenness, jointly indicate relative overgrowth of *S. aureus* in lesional skin and severe AD. Evenness was also associated with *C. acnes*; however, this correlation was positive. By the inherent definition of Evenness that it should penalize imbalanced species distributions, no microbial taxa should correlate *positively* with Evenness. This artefactual positive correlation between *C. acnes* and Evenness may be explained by the strong negative correlation between *S. aureus* and *C. acnes*, observed in our data as well as in previous research,[21,22,44] showing that *S. aureus* is central in the associations of other bacteria with Evenness in our data. Consistently, the lesional versus non-lesional differences in alpha diversity and the correlation of Evenness with disease severity vanished when *S. aureus* was excluded from the analysis. Overall, although other dominant species contributed to low Evenness values as well (Figure 1), and diversity measures were selected over *S. aureus* relative abundance in the regression models, *S. aureus* may be more easily measured in a clinical setting using quantitative PCR (qPCR) rather than microbiome Evenness, which requires sequencing of the whole microbiome.

Staphylococcus aureus is frequently proposed as a biomarker for AD severity.[26,45] However, as seen in our data as well as in previous research, the skin microbiome of AD patients is not always dominated by *S. aureus*.[46] In contrast to other studies,[43] skin sampling location could not explain the variation in *S. aureus* relative abundance and its association with AD severity. Apart from sampling location, different AD endotypes were proposed to contribute to differences between patient groups. It has been hypothesized that AD patients with low *S. aureus* abundance may potentially represent a distinct AD endotype.[38] In our data, race particularly affected the results of global microbiome composition and multifactorial regression. With substantial race-based differences observed in the microbiome of

healthy subjects,[47,48] and recently established racial endotypes found in transcriptome and immunologic data from AD patients,[6,49,50] racial endotypes might also explain differences in the AD skin microbiome. Unfortunately, the small sample size per race in our data does not allow for an in-depth analysis of individual races in AD. However, we suggest that racial subgroup analysis of larger microbiome datasets should investigate race-dependent patterns in the association between the skin microbiome and AD severity. This highlights the need for future studies, as to our knowledge no large multi-ethnic AD microbiome data is available for analysis to date.

Apart from microbiome Shannon or Evenness and *S. aureus* relative abundance, our multiple regression models indicate a strong influence of IgE levels on AD severity. The positive association of AD severity and total serum IgE levels has been observed in several other studies[51–53]; however, a meta-analysis did not recommend to use IgE as a single biomarker in AD.[54] Our analysis highlights the combined effect of IgE levels and *S. aureus* on AD severity. Although our regression models indicate the independent contribution of these two co-factors from a statistical perspective, it has been previously established that *S. aureus* can induce total serum IgE by the production of virulence factors such as δ-toxin,[55–57] supported by our observation that almost all patients with high *S. aureus* relative abundance also presented high IgE levels. In contrast, high IgE levels were also found in patients with low *S. aureus* relative abundance, suggesting that additional co-factors apart from *S. aureus* may contribute to high IgE levels and high AD severity. In consistence with that, we found evidence for the importance of other patient co-factors in these associations – such as sex, age, and race – which were previously found to be significant covariates both in AD and skin microbiome research.[2,26,27] These co-factors may be considered for explaining and measuring treatment success in clinical trials and highlight the need for the development of personalized AD treatments, especially when targeting the skin microbiome.[58]

Although our multiple regression models were overall statistically significant, they need to be validated in a larger study population, and only explained a maximum of 62% of variation in oSCORAD, supporting that other variables contributing to AD severity (e.g. filaggrin mutations affecting the skin barrier[19]) may be missing in our study.

Here, we broke down the concept of "low diversity" reported in AD and showed that severe AD is associated with an imbalanced skin microbiome distribution (low Evenness) rather than with a depletion of microbial taxa (low Richness). Our analyses validate the positive correlation between *S. aureus* relative abundance and AD severity in adults; however, we also find that other patient co-factors (IgE levels, age, sex, and particularly race) need to be considered in this association. Our exploratory analysis needs to be confirmed in larger data sets and validated by interventional studies to also provide mechanistic background of our findings on the associations between AD severity, microbiome diversity, and patient co-factors. Nevertheless, our results highlight the heterogeneity of AD and the need for larger cohorts to enable stratified analyses by patient demographics. Thus, as available treatment options are increasing,[58,59] our findings substantiate the need for personalized medicine in AD.

AUTHOR CONTRIBUTIONS

EGY and JK initiated and designed the clinical study. AUN, CTH, EGY and MR initiated and designed the microbiome analysis study. EGY and PMB managed the clinical investigation. MR managed the microbiome sequencing. MB performed the taxonomic annotation. AUN was responsible for data curation and guided data analysis. LR performed data analysis and prepared the figures. LR wrote the manuscript with significant contributions from AUN, and important input from MR, CTH, PMB, EGY and JK. CTH provided resources and support. All authors read and approved the final manuscript.

ACKNOWLEDGEMENTS

We thank Helmholtz Center Munich: Allergy-Projects, and Helmholtz Association, Germany: Impuls- und Vernetzungsfonds (IVF) for funding. We acknowledge IMGM Laboratories GmbH for help in sequencing the skin microbiome samples.

FUNDING INFORMATION

This work was supported by Helmholtz Center Munich: Allergy-Projects, and Helmholtz Association, Germany: Impuls- und Vernetzungsfonds (IVF).

CONFLICT OF INTEREST

The authors declare no conflict of interests for this article.

DATA AVAILABILITY STATEMENT

Microbiome raw sequencing data for this study have been deposited in the European Nucleotide Archive (ENA) at EMBL-EBI under accession number PRJEB58904 (https://www.ebi.ac.uk/ena/browser/view/PRJEB58904). Processed datasets and analysis scripts related to this article are available at https://github.com/LuiseRauer/Anti-IL22-baseline.

ETHICAL APPROVAL

The Icahn School of Medicine at Mount Sinai Institutional Review Board approved the study. All participants in this study provided written informed consent before inclusion.

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REFERENCES

1. Harrop J, Chinn S, Verlato G, Olivieri M, Norback D, Wjst M, et al. Eczema, atopy and allergen exposure in adults: a population-based study. Clin Exp Allergy. 2007;37(4):526–35.
2. Sacotte R, Silverberg JI. Epidemiology of adult atopic dermatitis. Clin Dermatol. 2018;36(5):595–605.
3. Simpson EL, Bieber T, Eckert L, Wu R, Ardeleanu M, Graham NM, et al. Patient burden of moderate to severe atopic dermatitis (AD): insights from a phase 2b clinical trial of dupilumab in adults. J Am Acad Dermatol. 2016;74(3):491–8.
4. Eckert L, Gupta S, Gadkari A, Mahajan P, Gelfand JM. Burden of illness in adults with atopic dermatitis (AD): analysis of National Health and Wellness Survey data from France, Germany, Italy, Spain and the U.K. J Am Acad Dermatol. 2019;81(1):187–95.
5. Odhiambo JA, Williams HC, Clayton TO, Robertson CF, Asher MI. Global variations in prevalence of eczema symptoms in children from ISAAC Phase Three. J Allergy Clin Immunol. 2009;124(6):1251–8. e23.
6. Czarnowicki T, He H, Krueger JG, Guttman-Yassky E. Atopic dermatitis endotypes and implications for targeted therapeutics. J Allergy Clin Immunol. 2019;143(1):1–11.
7. Bieber T, D'Erme AM, Akdis CA, Traidl-Hoffmann C, Lauener R, Schappi G, et al. Clinical phenotypes and endophenotypes of atopic dermatitis: where are we, and where should we go? J Allergy Clin Immunol. 2017;139(4S):S58–64.
8. Bakker DS, Nierkens S, Knol EF, Giovannone B, Delemarre EM, van der Schaft J, et al. Confirmation of multiple endotypes in atopic dermatitis based on serum biomarkers. J Allergy Clin Immunol. 2021;147(1):189–98.
9. Guttman-Yassky E, Waldman A, Ahluwalia J, Ong PY, Eichenfield LF. Atopic dermatitis: pathogenesis. Semin Cutan Med Surg. 2017;36(3):100–3.
10. Paller AS, Kong HH, Seed P, Naik S, Scharschmidt TC, Gallo RL, et al. The microbiome in patients with atopic dermatitis. J Allergy Clin Immunol. 2019;143(1):26–35.
11. Bjerre RD, Bandier J, Skov L, Engstrand L, Johansen JD. The role of the skin microbiome in atopic dermatitis: a systematic review. Br J Dermatol. 2017;177(5):1272–8.
12. Kong HH, Oh J, Deming C, Conlan S, Grice EA, Beatson MA, et al. Temporal shifts in the skin microbiome associated with disease flares and treatment in children with atopic dermatitis. Genome Res. 2012;22(5):850–9.
13. Totte JE, van der Feltz WT, Hennekam M, van Belkum A, van Zuuren EJ, Pasmans SG. Prevalence and odds of *Staphylococcus aureus* carriage in atopic dermatitis: a systematic review and meta-analysis. Br J Dermatol. 2016;175(4):687–95.
14. Byrd AL, Deming C, Cassidy SKB, Harrison OJ, Ng WI, Conlan S, et al. *Staphylococcus aureus* and *Staphylococcus epidermidis* strain diversity underlying pediatric atopic dermatitis. Sci Transl Med. 2017;9(397):eaal4651.
15. Leyden JJ, Marples RR, Kligman AM. *Staphylococcus aureus* in the lesions of atopic dermatitis. Br J Dermatol. 1974;90(5):525–30.
16. Polanska A, Danczak-Pazdrowska A, Silny W, Jenerowicz D, Olek-Hrab K, Osmola-Mankowska A. Nonlesional skin in atopic dermatitis is seemingly healthy skin – observations using noninvasive methods. Wideochir Inne Tech Maloinwazyjne. 2013;8(3):192–9.
17. Suarez-Farinas M, Tintle SJ, Shemer A, Chiricozzi A, Nograles K, Cardinale I, et al. Nonlesional atopic dermatitis skin is characterized by broad terminal differentiation defects and variable immune abnormalities. J Allergy Clin Immunol. 2011;127(4):954–64.e4.
18. Thukral A. A review on measurement of alpha diversity in biology. Agric Res J. 2017;54:1.
19. Clausen ML, Agner T, Lilje B, Edslev SM, Johannesen TB, Andersen PS. Association of disease severity with skin microbiome and filaggrin gene mutations in adult atopic dermatitis. JAMA Dermatol. 2018;154(3):293–300.
20. Gonzalez ME, Schaffer JV, Orlow SJ, Gao Z, Li H, Alekseyenko AV, et al. Cutaneous microbiome effects of fluticasone propionate cream and adjunctive bleach baths in childhood atopic dermatitis. J Am Acad Dermatol. 2016;75(3):481–93.e8.
21. Kwon S, Choi JY, Shin JW, Huh CH, Park KC, Du MH, et al. Changes in lesional and non-lesional skin microbiome during treatment of atopic dermatitis. Acta Derm Venereol. 2019;99(3):284–90.
22. Francuzik W, Franke K, Schumann RR, Heine G, Worm M. *Propionibacterium acnes* abundance correlates inversely with *Staphylococcus aureus*: data from atopic dermatitis skin microbiome. Acta Derm Venereol. 2018;98(5):490–5.
23. Shi B, Bangayan NJ, Curd E, Taylor PA, Gallo RL, Leung DYM, et al. The skin microbiome is different in pediatric versus adult atopic dermatitis. J Allergy Clin Immunol. 2016;138(4):1233–6.
24. Kim JE, Kim HS. Microbiome of the skin and gut in atopic dermatitis (AD): understanding the pathophysiology and finding novel management strategies. J Clin Med. 2019;8(4):444.
25. Bieber T, Traidl-Hoffmann C, Schäppi G, Lauener R, Akdis C, Schmid-Grendlmeier P. Unraveling the complexity of atopic dermatitis: the CK-CARE approach toward precision medicine. Allergy. 2020;75:2936–8.
26. Niemeyer-van der Kolk T, van der Wall HEC, Balmforth C, Van Doorn MBA, Rissmann R. A systematic literature review of the human skin microbiome as biomarker for dermatological drug development. Br J Clin Pharmacol. 2018;84(10):2178–93.
27. Szabo K, Erdei L, Bolla BS, Tax G, Biro T, Kemeny L. Factors shaping the composition of the cutaneous microbiota. Br J Dermatol. 2017;176(2):344–51.
28. Guttman-Yassky E, Brunner PM, Neumann AU, Khattri S, Pavel AB, Malik K, et al. Efficacy and safety of fezakinumab (an IL-22 monoclonal antibody) in adults with moderate-to-severe atopic dermatitis inadequately controlled by conventional treatments: a randomized, double-blind, phase 2a trial. J Am Acad Dermatol. 2018;78(5):872–81.e6.
29. Brunner PM, Pavel AB, Khattri S, Leonard A, Malik K, Rose S, et al. Baseline IL-22 expression in patients with atopic dermatitis stratifies tissue responses to fezakinumab. J Allergy Clin Immunol. 2019;143(1):142–54.
30. Callahan BJ, McMurdie PJ, Rosen MJ, Han AW, Johnson AJA, Holmes SP. DADA2: high-resolution sample inference from Illumina amplicon data. Nat Methods. 2016;13:581–3.
31. Bolyen E, Rideout JR, Dillon MR, Bokulich NA, Abnet CC, Al-Ghalith GA, et al. Reproducible, interactive, scalable and extensible microbiome data science using QIIME 2. Nat Biotechnol. 2019;37(8):852–7.
32. Bhattacharyya M, Reiger M, Rauer L, Huelpuesch C, Traidl-Hoffman C, Neumann AU. AnnotIEM: a novel algorithm for species level annotation of 16S gene based microbial OTUs [version 1; not peer reviewed]. F1000 Res. 2019;8:1660.
33. Cole JR, Wang Q, Fish JA, Chai B, McGarrell DM, Sun Y, et al. Ribosomal database project: data and tools for high throughput rRNA analysis. Nucleic Acids Res. 2014;42(Database issue):D633–42.
34. Oranje AP, Glazenburg EJ, Wolkerstorfer A, de Waard-van der Spek FB. Practical issues on interpretation of scoring atopic dermatitis: the SCORAD index, objective SCORAD and the three-item severity score. Br J Dermatol. 2007;157(4):645–8.
35. Schmitt J, Langan S, Deckert S, Svensson A, von Kobyletzki L, Thomas K, et al. Assessment of clinical signs of atopic dermatitis: a systematic review and recommendation. J Allergy Clin Immunol. 2013;132(6):1337–47.
36. R Core Team. R: A Language and Environment for Statistical Computing. Vienna, Austria: R Foundation for Statistical Computing; 2018.
37. Kunz B, Oranje AP, Labrèze L, Stalder JF, Ring J, Taïeb A. Clinical validation and guidelines for the SCORAD index: consensus report of the European Task Force on Atopic Dermatitis. Dermatology. 1997;195(1):10–9.
38. Fyhrquist N, Muirhead G, Prast-Nielsen S, Jeanmougin M, Olah P, Skoog T, et al. Microbe-host interplay in atopic dermatitis and psoriasis. Nat Commun. 2019;10(1):4703.
39. Hülpüsch C, Tremmel K, Hammel G, Bhattacharyya M, de Tomassi A, Nussbaumer T, et al. Skin pH-dependent *Staphylococcus aureus*

abundance as predictor for increasing atopic dermatitis severity. Allergy. 2020;75:2888–98.
40. Human Microbiome Project Consortium. Structure, function and diversity of the healthy human microbiome. Nature. 2012;486(7402):207–14.
41. Oh J, Conlan S, Polley EC, Segre JA, Kong HH. Shifts in human skin and nares microbiota of healthy children and adults. Genome Med. 2012;4(10):77.
42. Callewaert C, Nakatsuji T, Knight R, Kosciolek T, Vrbanac A, Kotol P, et al. IL-4Rα blockade by dupilumab decreases *Staphylococcus aureus* colonization and increases microbial diversity in atopic dermatitis. J Invest Dermatol. 2020;140(1):191–202.e7.
43. Ottman N, Barrientos-Somarribas M, Fyhrquist N, Alexander H, Wisgrill L, Olah P, et al. Microbial and transcriptional differences elucidate atopic dermatitis heterogeneity across skin sites. Allergy. 2021;76(4):1173–87.
44. Altunbulakli C, Reiger M, Neumann AU, Garzorz-Stark N, Fleming M, Huelpuesch C, et al. Relations between epidermal barrier dysregulation and *Staphylococcus* species-dominated microbiome dysbiosis in patients with atopic dermatitis. J Allergy Clin Immunol. 2018;142(5):1643–7.e12.
45. Reiger M, Traidl-Hoffmann C, Neumann AU. The skin microbiome as a clinical biomarker in atopic eczema: promises, navigation, and pitfalls. J Allergy Clin Immunol. 2020;145(1):93–6.
46. Ogonowska P, Gilaberte Y, Barańska-Rybak W, Nakonieczna J. Colonization with *Staphylococcus aureus* in atopic dermatitis patients: attempts to reveal the unknown. Front Microbiol. 2020;11:567090.
47. Leung MH, Wilkins D, Lee PK. Insights into the pan-microbiome: skin microbial communities of Chinese individuals differ from other racial groups. Sci Rep. 2015;5:11845.
48. Li M, Budding AE, van der Lugt-Degen M, Du-Thumm L, Vandeven M, Fan A. The influence of age, gender and race/ethnicity on the composition of the human axillary microbiome. Int J Cosmet Sci. 2019;41(4):371–7.
49. Brunner PM, Guttman-Yassky E. Racial differences in atopic dermatitis. Ann Allergy Asthma Immunol. 2019;122(5):449–55.
50. Kaufman BP, Guttman-Yassky E, Alexis AF. Atopic dermatitis in diverse racial and ethnic groups-variations in epidemiology, genetics, clinical presentation and treatment. Exp Dermatol. 2018;27(4):340–57.
51. Hon KL, Tsang KY, Kung JS, Leung TF, Lam CW, Wong CK. Clinical signs, *Staphylococcus* and atopic eczema-related seromarkers. Molecules (Basel, Switzerland). 2017;22(2):291.
52. Vaneckova J, Bukač J. The severity of atopic dermatitis and the relation to the level of total IgE, onset of atopic dermatitis and family history about atopy. Food Agric Immunol. 2016;27(5):734–41.
53. Laske N, Niggemann B. Does the severity of atopic dermatitis correlate with serum IgE levels? Pediatr Allergy Immunol. 2004;15(1):86–8.
54. Thijs J, Krastev T, Weidinger S, Buckens CF, de Bruin-Weller M, Bruijnzeel-Koomen C, et al. Biomarkers for atopic dermatitis: a systematic review and meta-analysis. Curr Opin Allergy Clin Immunol. 2015;15(5):453–60.
55. Nakamura Y, Oscherwitz J, Cease KB, Chan SM, Muñoz-Planillo R, Hasegawa M, et al. *Staphylococcus* δ-toxin induces allergic skin disease by activating mast cells. Nature. 2013;503(7476):397–401.
56. Simpson EL, Villarreal M, Jepson B, Rafaels N, David G, Hanifin J, et al. Patients with atopic dermatitis colonized with *Staphylococcus aureus* have a distinct phenotype and endotype. J Invest Dermatol. 2018;138(10):2224–33.
57. Patrick GJ, Liu H, Alphonse MP, Dikeman DA, Youn C, Otterson JC, et al. Epicutaneous *Staphylococcus aureus* induces IL-36 to enhance IgE production and ensuing allergic disease. J Clin Invest. 2021;131(5):e143334.
58. Wan P, Chen J. A calm, dispassionate look at skin microbiota in atopic dermatitis: an integrative literature review. Dermatol Ther (Heidelb). 2020;10(1):53–61.
59. van der Schaft J, Thijs JL, Garritsen FM, Balak D, de Bruin-Weller MS. Towards personalized treatment in atopic dermatitis. Expert Opin Biol Ther. 2019;19(5):469–76.

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

How to cite this article: Rauer L, Reiger M, Bhattacharyya M, Brunner PM, Krueger JG, Guttman-Yassky E, et al. Skin microbiome and its association with host cofactors in determining atopic dermatitis severity. J Eur Acad Dermatol Venereol. 2023;37:772–782. https://doi.org/10.1111/jdv.18776