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

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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 etiology 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 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 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 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 atopic dermatitis[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 **supplementary data**. 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 (**Supp. Table 1**). Cells were mechanically lysed and microbial DNA was extracted following the QIAamp UCP Pathogen Mini Kit (QIAGEN, Hilden, Germany) protocol. During PCR amplification, the hypervariable regions V1-V3 of the 16S rRNA gene were amplified. Samples were equimolarly pooled and analyzed together with positive and negative controls via multiplexed bidirectional sequencing (2x300 base pairs) using a MiSeq® system (Illumina, San Diego, CA). 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 less than 2,000 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 analyzed, 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 ($15 \leq$ oSCORAD ≤ 40) and severe (oSCORAD > 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 (**Fig. 1, Supp. Fig 1**) was dominated by *S. aureus*, which was detected in 79% of 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* ($r_S \geq 0.50$, $p < 0.001$) (**Supp. Fig. 2**).

The different skin locations sampled in this study (**Supp. Table 1**) were combined for analysis because we did not observe significant differences in the global microbiome composition between skin types (**Supp. Fig. 3a-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 Simpson diversity indices (which take the distinct alpha diversity component evenness into account), but not for Richness (**Fig. 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 (**Supp. Fig. 4a**). oSCORAD was also associated with the mixed alpha diversity measures Shannon and Simpson ($r_S < –0.52$, $p < 0.001$), but not with Richness ($r_S = –0.28$, $p = 0.07$) (**Fig. 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 status, the degree of overlap was investigated using Venn diagrams for all species present in at least 10% of lesional or non-lesional samples. We found that lesional and non-lesional samples shared all 183 species (**Supp. Fig. 5a**), and that high AD severity was not characterized by a reduced number of species in either skin status (**Supp. Fig. 5b**), 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 (**Fig. 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 ($r_S = -0.76$, $p < 0.001$) (**Supp. Fig. 6**). Similarly, these findings apply to non-lesional skin sites as well, although the associations were generally weaker (**Fig. 1, Supp. Fig. 6**).

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. Furthermore, excluding *S. aureus* removed all associations of alpha diversity with lesional versus non-lesional skin sites (**Supp. Fig. 7**), identifying *S. aureus* as the 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 ($p = 0.09$) (**Fig. 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 ($p = 0.04$) (**Fig. 3c-j**), whereas no significant variations in the microbiome composition were found between age or BMI groups ($p = 0.2$) (**Supp. Fig. 3c-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 (**Fig. 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. 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 ($r_S = 0.66$, $p < 0.001$) (**Fig. 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 race. Indeed, Asian participants tended to have slightly higher AD severity than African-American or Caucasian-American participants ($p = 0.12$, **Fig. 4c**). Analyzing the combined effects of both *S. aureus* relative abundance and IgE levels on oSCORAD (**Fig. 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 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 (**Supp. Fig. 4b**).

For non-lesional skin, the derived models from multiple regression were similar ($R^2 = 50\%$, $p < 0.001$) (**Supp. Table 2**) 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 **Supp. Fig. 8**, 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 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 (**Fig. 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.

S. 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 multi-factorial 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 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 datasets, 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.

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Data availability:

Microbiome raw sequencing data, processed datasets and analysis scripts related to this article can be found at https://osf.io/8s495/?view_only=9e9a7bfe83e24ea49e9d655a70f30947, hosted at the Open Science Framework platform (OSF).

Abbreviations:

AD: atopic dermatitis

ASV: amplicon sequence variant

BMI: body mass index

BSA: body surface area

IGA: Investigator Global Assessment

IgE: immunoglobulin E

LS: lesional skin

NL: non-lesion skin

nMDS: non-metric multidimensional scaling

n.s.: not (statistically) significant

(o)SCORAD: (objective) scoring atopic dermatitis

PERMANOVA: permutational analysis of variance

Rel. abundance: relative abundance

SD: standard deviation

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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.

Ethics 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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TABLES

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

	All (n = 48)	Moderate AD† (n = 17)	Severe AD† (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‡, mean (SD)	27.3 (6.2)	27.6 (5.8)	27.2 (6.5)	0.58
Sex, n				0.55
Female	22 (46%)	9 (53%)	13 (42%)	
Male	26 (54%)	8 (47%)	18 (58%)	
Race, n				0.28
Asian-American	12 (25%)	2 (12%)	10 (32%)	
African-American	21 (44%)	8 (47%)	13 (42%)	
Caucasian	15 (31%)	7 (41%)	8 (26%)	
IgE group, n				0.13
Intrinsic (IgE < 200 kU/l)	10 (21%)	6 (35%)	4 (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

†AD severity was defined by objective SCORAD (moderate: 15 ≤ oSCORAD ≤ 40, severe: oSCORAD > 40).

‡BMI values missing for two patients.

P-values were obtained by the Mann-Whitney U test for continuous variables and Fisher's exact test for categorical variables.

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

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)**

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

FIGURE LEGENDS

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 ten most abundant species. Samples in lesion and non-lesion are ordered paired by subjects, according to ascending Evenness in lesion.

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.

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 vs. extrinsic IgE levels (**c, d**), moderate vs. 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 1,000 permutations and ellipses denote 95% confidence intervals around cluster centroids. Bar plots show mean microbiome composition of the ten most abundant species (**b, d, f, h, j**). oSC: objective scoring atopic dermatitis. LS: lesional skin, NL: non-lesional skin.

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 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 outliers with low *S. aureus* relative abundance and high AD severity. LS: lesional skin, rs: Spearman's rank correlation coefficient rho.

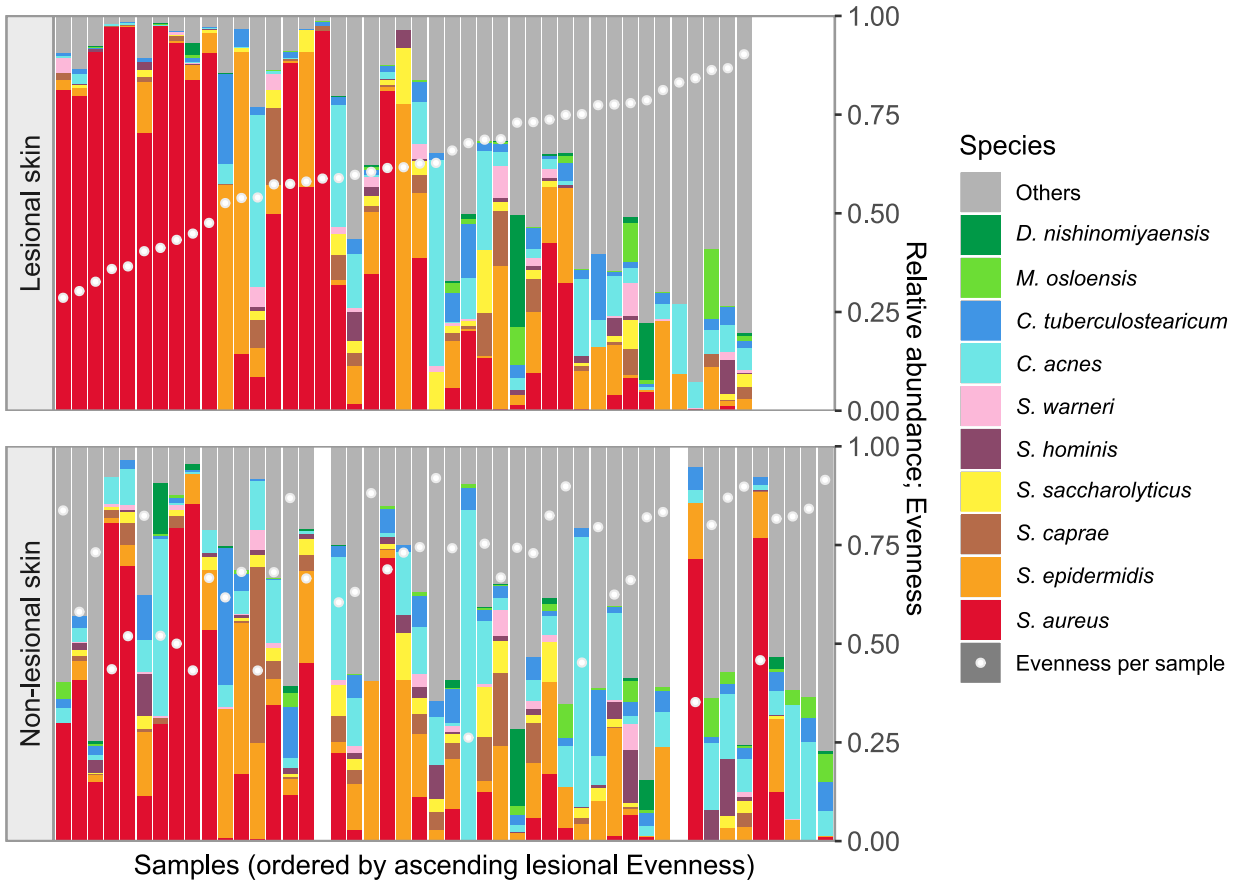

Figure 1

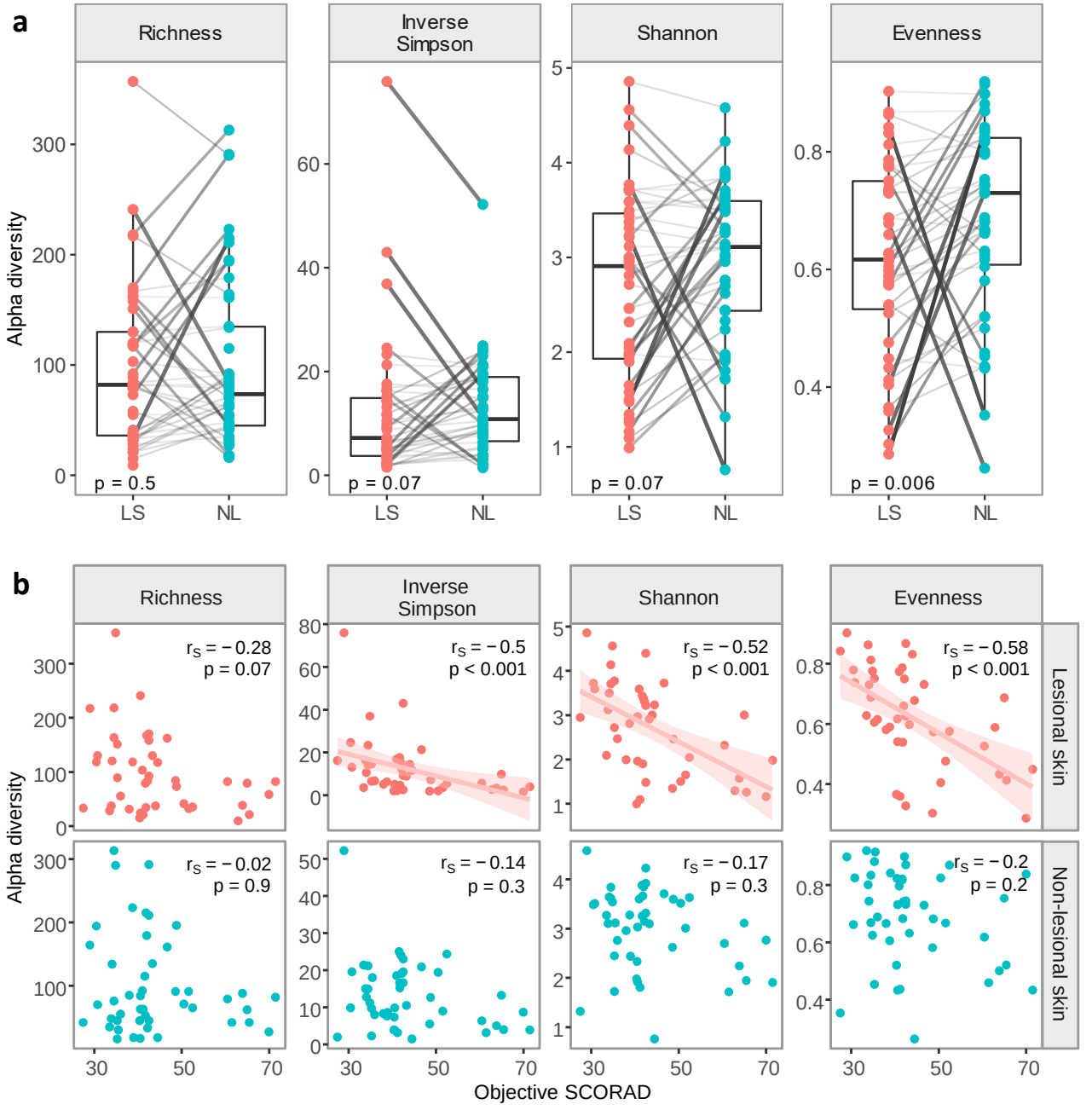

Figure 2

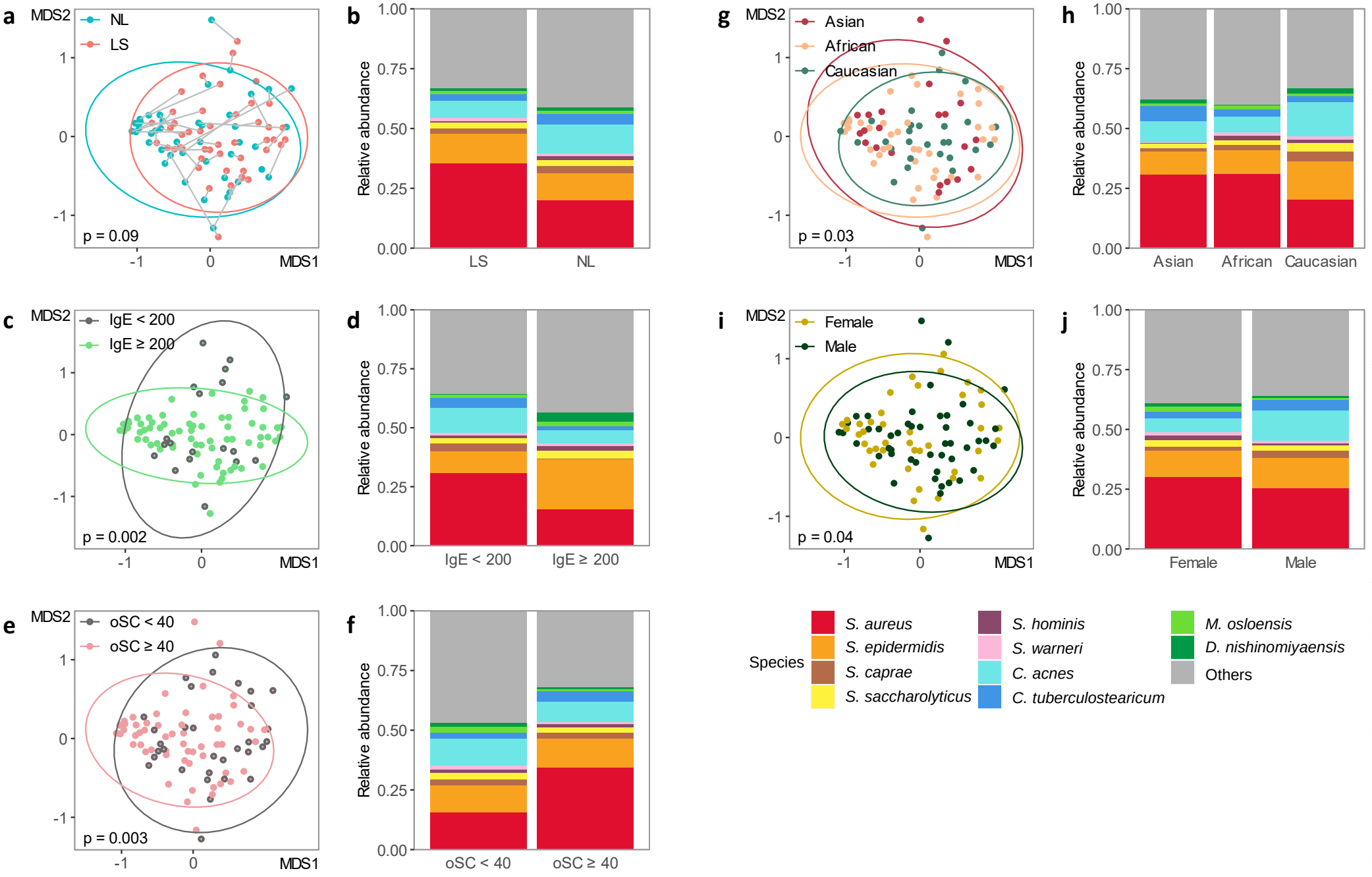

Figure 3

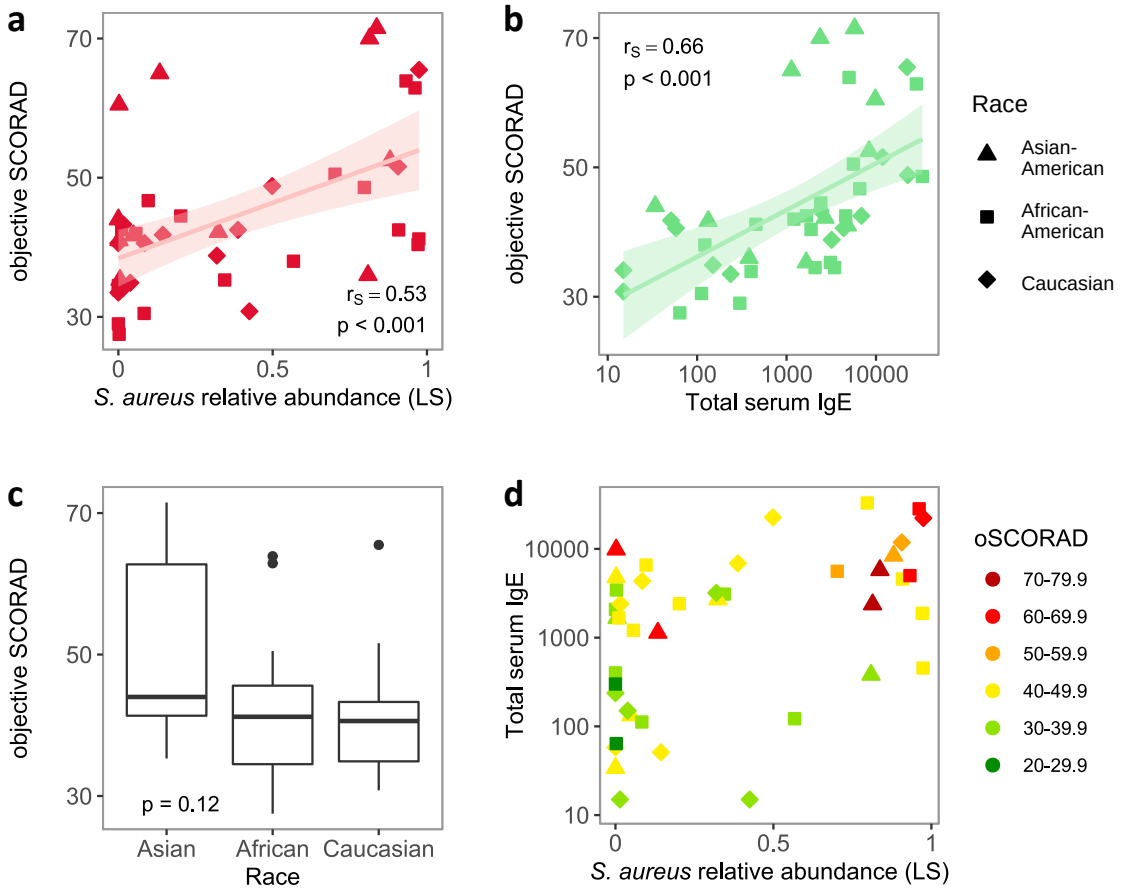

Figure 4