Is cutaneous microbiota a player in disease pathogenesis? Comparison of cutaneous microbiota in psoriasis and seborrheic dermatitis with scalp involvement

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Abstract

Background: Knowledge about cutaneous microbiota in psoriasis vulgaris and seborrheic dermatitis is limited, and a comparison of microbiota in the two diseases was not yet previously undertaken.

Aims/Objectives: This study aimed to compare the scalp lesional and non-lesional microbiota in psoriasis vulgaris and seborrheic dermatitis with that in a healthy control group.

Methods: Fifty samples were taken with sterile swabs from patients' and controls' scalps, and 16S rRNA gene sequencing analyses were performed.

Results: Alpha and beta diversity analyses showed that bacterial load and diversity were significantly increased in psoriasis vulgaris and seborrheic dermatitis lesions compared to the controls. As phyla, *Actinobacteria* decreased and *Firmicutes* increased, while as genera, *Propionibacterium* decreased; *Staphylococcus*, *Streptococcus, Aquabacterium*, *Neisseria* and *Azospirillum* increased in lesions of both diseases. Specifically, *Mycobacterium*, *Finegoldia*, *Haemophilus* and *Ezakiella* increased in psoriasis vulgaris and *Enhydrobacter*, *Micromonospora* and *Leptotrichia* increased in seborrheic dermatitis lesions. *Mycobacterium*, *Ezakiella* and *Peptoniphilus* density were higher in psoriasis vulgaris compared to seborrheic dermatitis lesions. The bacterial diversity and load values of non-lesional scalp in psoriasis vulgaris and seborrheic dermatitis lay between those of lesional areas and controls.

Limitations: The small sample size is the main limitation of this study.

Conclusion: Higher bacterial diversity was detected in lesions of both psoriasis and seborrheic dermatitis compared to the controls, but similar alterations were observed when the two diseases were compared. Although these differences could be a result rather than a cause of the two diseases, there is a need to analyze all members of the microbiota and microbiota-host interactions.

Key words: Chronic inflammatory disease, cutaneous microbiota, psoriasis vulgaris, scalp, seborrheic dermatitis

Plain Language Summary

Seborrheic dermatitis and psoriasis vulgaris are inflammatory skin disorders that may affect the scalp. We aimed to investigate the differences in the scalp bacterial microbiota in these diseases and compare the results with healthy examples. Fifty swab samples were taken from both the lesions and the lesion-free scalp of the patients and the control groups. Analyzing 16S rRNA regions of the examples were used for bacterial identification. Alpha and beta diversity analyses showed that bacterial load and diversity significantly increased in psoriasis vulgaris and seborrheic dermatitis lesions compared to the control. *Actinobacteria*, *Firmicutes*,

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and *Proteobacteria* were the three phyla dominating the overall community in all five groups. *Actinobacteria* decreased in both lesional and lesion-free examples compared to the control, while there was a significant abundance in *Firmicutes* in disease groups. As genus, *Propionibacterium* decreased, but *Staphylococcus*, *Streptococcus*, *Aquabacterium*, *Neisseria*, and *Azospirillum* increased in lesions of both diseases. The skin microbiota values of the lesion-free disease samples were between those of the healthy and disease lesional groups in both diseases. The two disorders were observed to share very similar microbiota compositions and diversity. The bacterial similarities between the two diseases suggest that these differences are a result rather than a cause.

Introduction

The symbiotic relationship between the host and the cutaneous microbial communities helps protect the body against external factors.[1] The community composed of microorganisms on the skin is called the cutaneous microbiota.[2] The cutaneous microbiota prevents colonization and invasion by pathogenic microorganisms and educates the host's immune system against bacteria that may be pathogenic.[3-5]

There are different microorganisms with different densities in every region of the skin. The distribution of the cutaneous microbiota may be considered under three regions: Dry areas such as the legs and back, moist areas such as the axillae and groins, and sebaceous areas such as the face and scalp.[6] *β-Proteobacteria* and *Flavobacteriales* are mostly found in dry areas. In humid parts, mostly Corynebacteria and rarely Staphylococci species are seen. Propionibacteria and Staphylococci species are found in sebaceous regions.[7] The four main bacterial phyla found on the skin are *Actinobacteria* (52%), *Firmicutes* (24%), *Proteobacteria* (17%) and *Bacteroidetes* (7%).[7,8]

This symbiotic relationship may be disrupted, leading to pathogenicity in some cases.[9] The relationship between the microbiota and skin disorders such as acne vulgaris, skin cancers, hidradenitis suppurativa and especially atopic dermatitis and rosacea has been previously studied.[10-14] Only a little is specifically known about the scalp microbiota.[15,16] Seborrheic dermatitis is an inflammatory skin disorder that affects the scalp and other sebum-rich areas. Although its aetiopathogenesis is not fully understood, it is thought that free fatty acids such as oleic acid formed by the breakdown of sebum by *Malassezia* spp. initiate an inflammatory response and cause epidermal hyperproliferation.[17-19]

Psoriasis vulgaris is a T-cell-mediated chronic inflammatory skin disorder that frequently affects the scalp. In recent years, it has been suggested that dysbiosis of the skin microbiota may be associated with this condition.[20,21] An increase in *Malassezia* spp. on the scalp of psoriasis patients has been shown, compared to healthy individuals.[22,23] *Malassezia* spp. are considered to exacerbate psoriasis by causing complement activation, inducing the cytokines, and increasing neutrophil numbers locally.[23]

We aimed to investigate the differences in the skin bacterial microbiota in these two inflammatory skin disorders affecting the scalp which have clinical similarities, and to compare the results with those in a healthy control group.

Methods

Patients

The diagnosis was established in each case by checking the patient's file who were followed up and by confirming their disease with clinical examination. Patients with well-defined erythematous plaques covered by silvery-white scales on elbows, knees and other parts of the body were examined for scalp involvement by psoriasis vulgaris. Typical lesions on the scalp were taken as psoriasis vulgaris of the scalp. Poorly defined, salmon-coloured plaques with a greasy, yellowish scale on the seborrheic areas such as scalp and face were considered as seborrheic dermatitis. The diagnosis was confirmed in each case by a dermatologist and was rechecked by other dermatologists. If the dermatologists agreed on the diagnosis, the patient was recruited.

The controls were obtained from healthy volunteers who came to the outpatients' clinic for cosmetic concerns such as hyperpigmentation, wrinkles, sun protection etc. and who had no lesions on the scalp.

Procedure

The study included consenting patients diagnosed with psoriasis vulgaris and seborrheic dermatitis and healthy volunteers (mean ages, 45.9, 48.1, and 47.7 years, respectively). The cases and controls were matched for age. Each group had five female and five male participants who had used no treatment (including topicals, medicated shampoos, antibiotics or any other medications) for the last two months, who had no other chronic diseases, and who had not taken a bath for at least 24 h before sample collection. Each of the disease groups and the control group had 10 subjects. So we had a total of 30 patients and 50 swabs. (20 swab samples were taken from the lesions, 20 swab samples were taken from the non-lesional parts of the scalp and 10 swab examples were taken from the healthy group).

The psoriasis patients had never received systemic psoriasis treatment. Psoriasis area and severity index (PASI) and seborrheic dermatitis area and severity index (SEDASI) were used to score the severity of the diseases. The average psoriasis area and severity index score was 9.75 ± 6.39 (range, 3.5-24.3) for psoriasis vulgaris patients, while the average seborrheic dermatitis area and severity index score was 17.3 ± 8.83 (range, 5-28) for patients with seborrheic dermatitis.

The study was planned as a preliminary study, and the sample size of the groups was the least required number for significant statistical analysis. The study approval was obtained from the Istanbul Medeniyet University ethics committee (15 August 2018/0310). The study protocol was registered at https://www.clinicaltrials.gov with the number NCT03807453.

Sample collection and preservation

Fifty swab samples were taken from both the lesions and lesion-free hairy scalp of the patients and the scalp of controls. Ten swab samples each were taken from the lesions of psoriasis,

the lesions of seborrheic dermatitis, the non-lesional parts of psoriais, the non-lesional parts of seborrheic dermatitis and from the healthy group. A single investigator (M.A.K.) selected the sampling sites and collected the samples from all the cases and controls. Sterile swabs were placed inside DNA/RNA Shield™ collection tube w/swab tubes. Samples were taken with different swabs from the lesions and lesion-free areas from the scalps of the same patients. The investigators usually tried to collect the samples from the controls from the same areas, usually the parietal area or vertex.The samples were taken by rubbing swabs soaked with sterile distilled water on the area for one minute. The swabs were immediately put in sterile solution, containing DNA/RNA Shield, inside closed tubes at -20°C until microbiota analysis was performed.

16S rRNA gene sequencing

In order to reveal the bacterial compositions of the samples, Amplicon-based sequencing targeting the 16S rRNA regions, which are used for bacterial identification and classification, was performed. For that purpose, ZymoBIOMICS targeted sequencing service for microbiome analysis (Zymo Research, USA) was used for DNA isolation and sequencing. The V3-V4 region of the 16S ribosomal RNA (rRNA) gene was targeted, and the prepared library was sequenced on the Illumina MiSeq sequencing platform in paired-end reading mode.

Bioinformatical analysis

Bioinformatical analysis was carried on the FASTQ reads obtained from the sequencing analysis. There were 2,131,964 reads obtained for 50 samples. These reads were imported into the quantitative insights into microbial ecology (Qiime2) software pipeline, version 2020.2, for analysis.[24] The sequences were demultiplexed using the "demux" plugin. DADA2 was chosen for quality filtering, trimming of adapters, denoising, error removal, and merging of the paired-end sequences. The sequences were rarefied to an equal depth of 8,350 sequences per sample, which retained 43.6% of features in all 50 samples. For taxonomy assignment, the 16S rRNA sequence database from bacteria and archaea was obtained from NCBI. The "q2-feature-classifier" plugin with the naïve Bayes classifier was trained on the V3-V4 region of the database. The sequences assigned to "eukaryote" and "mitochondria" and those that were "unassigned" were filtered out before proceeding to the downstream analysis.

Alpha and beta diversity analyses

The diversity within a sample is called alpha diversity.[25] Three common alpha diversity measures were used: the number of observed operational taxonomic units reflecting the richness (how many different bacteria are present in the sample) only, Shannon's diversity index providing equal weight to richness and evenness (how evenly are these bacterial taxa distributed within the sample), and Faith's phylogenetic diversity index accounting for relationships based on phylogenetics.[26]

Beta diversity shows the variety of different bacteria among the samples. The principal component analysis with the unweighted or weighted UniFrac distance analysis was used. As the phylogenetic beta diversity measurement, the UniFrac measure with two types, weighted (qualitative) (considering abundance) and unweighted (quantitative) (considering presence or absence of bacterial taxa) analyses, were conducted.[27] By this way, clustering patterns between the different patient groups were examined to reveal if any inter-group differences exist.

Statistical analysis

Relative frequency data at the taxonomic level 2 (phylum) and 6 (genus), alpha and beta diversity metrics were exported from Qiime2. All statistical analyses and visualizations were carried using R (version 3.6.1). The Shapiro-Wilk test was used to test the distribution normality. For the alpha diversity analysis, one-way analysis of variance followed by the post hoc Tukey's honest significant difference test, or the Kruskal-Wallis analysis of variance comparison followed by the posthoc Dunn's test were applied for the normally and non-normally distributed data, respectively. The permutational multivariate analysis of variance test was performed using Qiime2 to determine the significance of differences in beta-diversity distances between the compared groups. The differences in the relative abundance of taxa between the groups were tested using the Mann-Whitney U test.

Results

Psoriasis vulgaris

The alpha diversity analysis is shown in Figures 1a-1c. The skin microbiota values of the non-lesional psoriasis vulgaris samples lay between those of the healthy control and psoriasis lesional groups, but there was a statistical difference only between the lesional and control groups. The beta diversity analysis also revealed different clustering for the control and lesional psoriasis vulgaris samples [Figures 1d-1g]. On the other hand, the comparison of the lesional and non-lesional psoriasis vulgaris samples showed overlapping clustering. The pairwise comparison using the permutational multivariate analysis of variance test revealed a significant difference between the control and lesional group (unweighted: pseudo-F = 2.73, q = 0.003; weighted: pseudo-F = 5.29, q = 0.045), while no significant difference was detected between the lesional and non-lesional samples of psoriasis. (Unweighted: pseudo-F = 1.18, q = 0.27; weighted: pseudo-F = 0.9, q = 0.57).

To reveal the differences in the bacterial composition of the control and psoriasis vulgaris groups, phylum and genus-based comparisons were carried out [Figure 2]. *Actinobacteria, Firmicutes* and *Proteobacteria* were the three phyla dominating the overall community in both healthy and psoriasis skin microbiota [Figure 3]. However, the balance between these three groups was determined to be disturbed in the psoriasis vulgaris microbiota. The relative abundance of *Actinobacteria* decreased in both lesional and non-lesional psoriasis groups compared to the control [Figure 3a]. The differences in the relative abundance of *Proteobacteria* were not significant [Figure 3b]. The pairwise comparisons of the psoriatic lesional and non-lesional microbiota compositions at the phylum level did not show any significant difference.

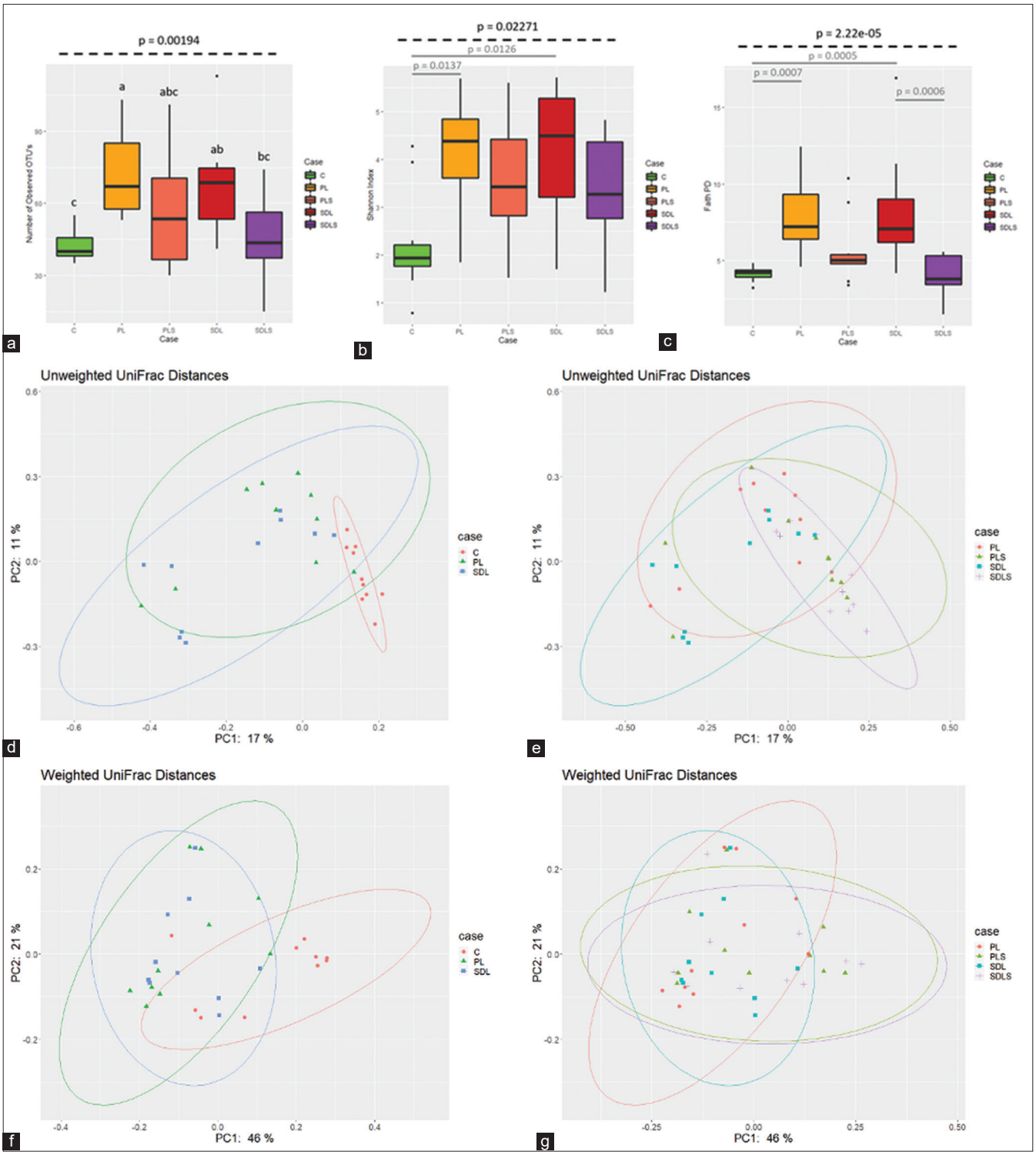


Figure 1: Alpha diversity analysis using (a) observed operational taxonomic unit: (b) Shannon's diversity index and (c) Faith's phylogenetic diversity index, compared between the case groups. Differences in observed operational taxonomic unit across the case groups were compared using the one-way analysis of variance (top dashed line) and post hoc Tukey's honest significant difference test (Different superscript letters indicate significant differences, $P < 0.05$) while Shannon's diversity and Faith's phylogenetic diversity indices were compared using the Kruskal-Wallis analysis of variance (top dashed line) and post hoc Dunn's test (grey solid lines). Beta diversity analysis using principal component analysis plots based on unweighted (d and e) and weighted (f and g) Unifrac distances. Ellipses correspond to the 95% confidence intervals for each case group. OTU: Operational taxonomic unit, C: Control, PL: Psoriasis vulgaris lesional site, PLS: Psoriasis vulgaris non-lesional site, SDL: Seborrheic dermatitis lesional site, SDLS: Seborrheic dermatitis non-lesional site

However, there was a significant increase in the abundance of *Firmicutes* in the lesional and non-lesional psoriasis groups when compared to the controls [Figure 3c].

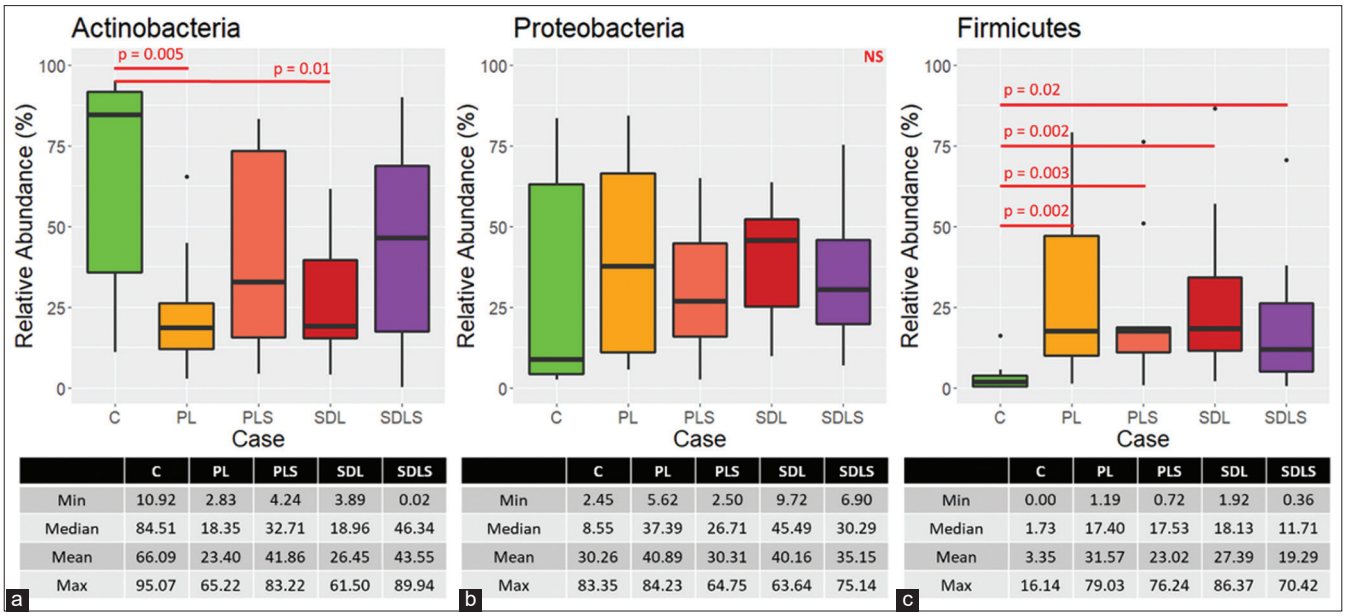
The comparison of the control and psoriasis vulgaris groups showed substantial alterations in some genera. In the lesional psoriasis samples, there was a statistically significant decrease

Figure 2: Most abundant phyla (top) and genera (bottom) across the case groups. The median relative abundance of the three most abundant phyla and ten most abundant genera are used for the illustration

in the *Propionibacterium* levels and a significant increase in the levels of *Staphylococcus*, *Streptococcus*, *Neisseria*, *Mycobacterium*, *Finegoldia*, *Haemophilus*, *Azospirillum* and *Ezakiella* [Figure 4]. The comparison between the non-lesional and lesional psoriasis vulgaris groups showed an increase in the *Nocardioides* and *Anaerococcus* abundance in the lesional group. In addition, when compared with the control group, *Staphylococcus*, *Streptococcus* and *Haemophilus* abundances were shown to be increased in the lesional samples.

Seborrheic dermatitis

The alpha diversity analysis revealed enhanced richness and evenness in seborrheic dermatitis, and the difference was more prominent and statistically significant in the lesional group. The diversity indices for the non-lesional seborrheic dermatitis samples lay between the values of the control and lesional seborrheic dermatitis groups, but the difference between the lesional and non-lesional samples was statistically significant only according to Faith's phylogenetic diversity analysis [Figures 1a-1c]. The beta diversity analysis also confirmed the significant diverse clustering of the lesional seborrheic dermatitis group compared to the controls (unweighted: pseudo-F = 3.42, q = 0.003; weighted: pseudo-F = 6.43, q = 0.02) [Figures 1d-1g]. When the lesional and non-lesional groups of seborrheic dermatitis were compared with the beta diversity analysis, the unweighted UniFrac distance revealed a significant difference (unweighted: pseudo-F = 2.69, q = 0.003; weighted: pseudo-F = 1.3, q = 0.45).



	C	PL	PLS	SDL	SDLS
Min	10.92	2.83	4.24	3.89	0.02
Median	84.51	18.35	32.71	18.96	46.34
Mean	66.09	23.40	41.86	26.45	43.55
Max	95.07	65.22	83.22	61.50	89.94

a

	C	PL	PLS	SDL	SDLS
Min	2.45	5.62	2.50	9.72	6.90
Median	8.55	37.39	26.71	45.49	30.29
Mean	30.26	40.89	30.31	40.16	35.15
Max	83.35	84.23	64.75	63.64	75.14

b

	C	PL	PLS	SDL	SDLS
Min	0.00	1.19	0.72	1.92	0.36
Median	1.73	17.40	17.53	18.13	11.71
Mean	3.35	31.57	23.02	27.39	19.29
Max	16.14	79.03	76.24	86.37	70.42

c

Figure 3: Comparison of the relative per cent abundances of the three dominant phyla, (a) *Actinobacteria*, (b) *Proteobacteria* and (c) *Firmicutes*, between the case groups. The differences between the two case subgroups were evaluated using the Mann-Whitney U test. The respective p values for the significant results are given in the figures. The differences observed in *Proteobacteria* were determined to be non-significant. C: Control, PL: Psoriasis vulgaris lesional site, PLS: Psoriasis vulgaris non-lesional site, SDL: Seborrheic dermatitis lesional site, SDLS: Seborrheic dermatitis non-lesional site

The differences in the bacterial composition of the control and seborrheic dermatitis groups with phylum and genus-based comparisons are shown in Figure 2. *Actinobacteria*, *Proteobacteria* and *Firmicutes* were the three dominant phyla in the disease group [Figure 3]. The pairwise comparisons of the lesional seborrheic dermatitis and controls revealed a significant decrease in *Actinobacteria* [Figure 3a] and an abundance in *Firmicutes* [Figure 3c]. The increase in *Firmicutes* was also significant in the non-lesional group compared to the controls. The differences in *Proteobacteria* were not significant [Figure 3b]. There were also no statistically significant differences in the abundances between the lesional and non-lesional seborrheic dermatitis groups at phylum level.

The genus-based differences are illustrated in Figure 4. Compared to control, the only decrease in the lesional seborrheic dermatitis group was seen in *Propionibacterium*, and increased genera detected were *Micromonospora*, *Staphylococcus*, *Streptococcus*, *Leptotrichia*, *Azospirillum*, *Aquabacterium*, *Neisseria* and *Enhydrobacter*. There were no significant differences in the pairwise comparison of the control and non-lesional groups and that of the lesional and non-lesional seborrheic dermatitis groups at the genus level.

Psoriasis vulgaris and seborrheic dermatitis

The alpha diversity measurements showed similar richness in the lesional psoriasis vulgaris and seborrheic dermatitis groups, as well as the non-lesional groups [Figures 1a-1c]. Similarly, the UniFrac measures showed overlapping diversity shared by the lesional or non-lesional psoriasis vulgaris as well as seborrheic dermatitis microbiota [Figures 1d-1g], (lesional psoriasis vulgaris and lesional seborrheic dermatitis: unweighted: pseudo-F = 1.44, q = 0.097; weighted: pseudo-F = 0.37, q = 0.95, and non-lesional psoriasis vulgaris and non-lesional seborrheic dermatitis: unweighted: pseudo-F=1.44, q = 0.11; weighted: pseudo-F = 0.21, q=0.97). A significant difference was obtained from the comparison of the lesional psoriasis vulgaris and seborrheic dermatitis groups using the unweighted UniFrac analysis (unweighted: pseudo-F = 2.36, q = 0.008; weighted: pseudo-F = 1.27, q = 0.45).

Phylum- and genus-based comparisons of the psoriasis vulgaris and seborrheic dermatitis groups are shown in Figure 2. When the differences at the phylum level were compared between the two disease groups, no significant difference was found in lesional or non-lesional regions [Figure 3]. The comparison of the lesional disease groups at the genus level revealed that three distinct taxa, *Mycobacterium*, *Ezakiella* and *Peptoniphilus* were only present in psoriasis vulgaris [Figure 4]. An increased abundances of *Nocardioides* and *Aquabacterium* were observed in non-lesional seborrheic dermatitis when compared with non-lesional psoriasis vulgaris regions [Figure 4].

Discussion

Microbiota alterations in psoriasis vulgaris and seborrheic dermatitis have been examined and reported in many studies [Tables 1 and 2]; however, microbiota differences between the two diseases have not been previously investigated.[21,28-40]

Both alpha and beta diversity indices, measuring the bacterial diversity within and between samples respectively, indicated similarly increased bacterial diversity in both diseases with respect to the controls. The microbiota compositions of the lesional samples of both psoriasis vulgaris and seborrheic dermatitis also significantly differed from the controls. As in our study, both richness and evenness of the alfa diversity of the seborrheic dermatitis lesional areas were found to be high

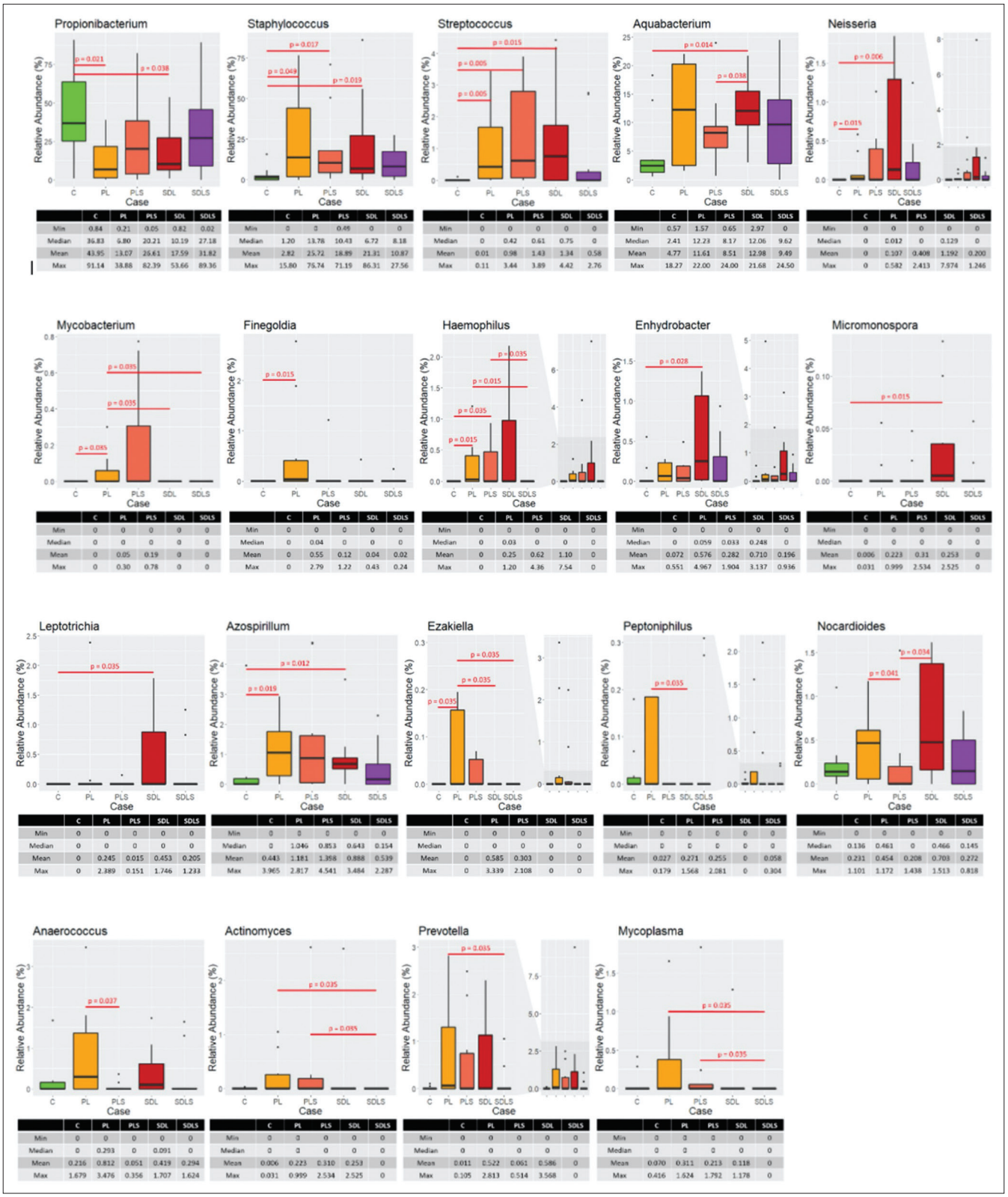


Figure 4: Relative abundance of the genera of interest that were determined to significantly differ between the case groups. The pairwise comparisons were carried out using the Mann-Whitney U test for all case groups. The minimum (min), mean, median and maximum (max) per cent abundances in the respective case groups are presented in the charts under each genus plot

in a study.[29] The alpha diversity of psoriasis vulgaris lesional samples was reported to be high in some studies, as in our study, but the alpha diversity of our control group was higher compared to previous reports.[30,36] Beta diversity in psoriasis was shown to be high in previous studies, consistent with our findings.[30,33] A remarkable observation obtained from

Table 1: Microbiota differences related to psoriasis vulgaris in previous studies

Study team/ year/country	Number of patients	Sampling and analysis method	Regions sampled	Most abundant bacteria		
				Lesional	Non-lesional	Control
Fahlén *et al*.[21] 2012 Sweden	Psoriasis 10 Control 12	Skin biopsy, 16S rRNA gene and the variable regions V3–V4	Trunk and limbs	Phylum: *Firmicutes* 39% Genus: *Staphylococcus* 5%, *Streptococci* 32% Phylum: *Proteobacteria* 38% *Actinobacteria* 5%	None	Phylum: *Firmicutes* 43% Genus: *Staphylococcus* 16%, *Streptococci* 26% Phylum: *Proteobacteria* 27% *Actinobacteria* 16%
Gao *et al* [28] 2008 USA	Psoriasis 6 Control 6	Swab, 16S rDNA PCR sequences	Usually forearm for controls and non-lesional samples Usually limbs for lesional samples	Phylum: *Firmicutes* 46.2% Genus: *Streptococcus* 15.2% *Staphylococcus* 18.8% Phylum: *Actinobacteria* 37.3% Genus: *Propionibacterium* 2.9% Phylum: *Proteobacteria* 11.4%	Phylum: *Firmicutes* 39% Genus: *Streptococcus* 3.4% Phylum: *Actinobacteria* 47.8% Genus: *Propionibacterium* 12.3% Phylum: *Proteobacteria* 10.1%	Phylum: *Firmicutes* 24.4% Genus: *Streptococcus* 3.7% Phylum: *Actinobacteria* 47.6% Genus: *Propionibacterium* 21.1% Phylum: *Proteobacteria* 21.9%
Alekseyenko *et al*.[29] 2013 USA	Psoriasis 54 Control 37	Swab, 16S rRNA gene amplification	Patient group: uncertain Controls: scalp, forearm, abdomen, kneecap	Phylum: *Proteobacteria* 30.75% Genus*: Corynebacterium*, *Streptococcus* and *Staphylococcus* combination: 33.8%	Phylum: *Proteobacteria* 36.21% Genus: *Corynebacterium, Streptococcus* and *Staphylococcus* combination: 22.9%	Phylum: *Proteobacteria* 33.32% Genus: *Corynebacterium, Streptococcus* and *Staphylococcus* combination: 22.03%
Chang *et al*.[30] 2018 USA	Psoriasis 28 Control 26	Swab, 16S rRNA V1–V3 variable region	Patient group: frequently arms, legs, and scalp Controls: scalp, trunk, arm, leg, axilla, and gluteal fold	Phylum: *Actinobacteria>Firmicutes >Proteobacteria* Genus: *Staphylococcus aureus >Staphylococcus pettenkoferi*	Phylum: *Actinobacteria>Firmicutes>Proteobacteria* Genus: *Staphylococcus sciuri*	Phylum: *Actinobacteria>Firmicutes>Proteobacteria* Genus: *Propionibacterium acnes>Propionibacterium granulosum*
Tett *et al*.[31] 2017/Italy	28	Swab, high-resolution shotgun metagenomics (DNA sequencing)	Elbow Retroauricular fold	Phylum: *Actinobacteria* and *Firmicutes* Genus: *Staphylococcus caprae/capitis* *Staphylococcus aureus* *Staphylococcus epidermidis* *Micrococcus luteus*	Phylum: *Actinobacteria* and *Firmicutes* Genus: *Propionibacterium acnes*	None
Tomi *et al*.[32] 2005 Austria*	Psoriasis 25 Control 25	Swab, Bacterial culture	Uncertain	*S. aureus* 60%	None	*S. aureus* 4%
Quan *et al*.[33] 2020 China	Psoriasis 27 Control 19	Swab, 16S rRNA sequencing	Dry and sebaceous site (not scalp)	Phylum: *Bacteroidetes* 1.83% Deinococcus-Thermus 0.13% Genus *Propionibacterium* 0.15% *Corynebacterium* 7.98%	Phylum: *Bacteroidetes* 3.94% Deinococcus-Thermus 0.25% Genus *Propionibacterium* 2.52% *Corynebacterium* 6.04%	Phylum: *Bacteroidetes* 5.06% Deinococcus-Thermus 0.45% Genus *Propionibacterium* 8.88% *Corynebacterium* 3.36%
Loesche *et al*.[34] 2018 USA (Ustekinumab Phase3b study)	114	Swab, 16S rRNA gene	Arm, axilla, buttock, leg, scalp, and trunk	Phylum: Leg, scalp, and trunk lesions: high *Actinobacteria*. Scalp and trunk lesions: low *Firmicutes* Genus: Leg, non-lesional: *Caulobacteraceae* and *Corynebacterium* Scalp, lesional: *Bacilli* Scalp, non-lesional: *Propionibacterium acnes*		None
Martin *et al*.[35] 2015 France	29	Swab, 16S rRNA genes V1-V2 region	Uncertain	Phylum: *Actinobacteria* 28% Genus: *Corynebacterium* 13% *Propionibacterium* 7% Phylum: *Firmicutes* 42% Genus: *Staphylococcus* 18% Phylum: *Proteobacteria* 22%	Phylum: *Actinobacteria* 32% Genus: *Corynebacterium* 11% *Propionibacterium* 9% Phylum: *Firmicutes* 39% Genus: *Staphylococcus* 16% Phylum: *Proteobacteria* 21%	None

*Only *Staphylococcus aureus* was studied in this study

Table 2: Microbiota differences related to seborrheic dermatitis in previous studies

Study team/ year/country	Number of Patients			Sampling and analysis methods	Regions sampled	Most colonized bacteria		
						Lesional	Non-lesional	Control
Tanaka *et al*.[36] 2016 Japan	24			Squame sampling 16S rRNA gene	Ala nasi	Genus: *Staphylococcus, Acinetobacter*, *Streptococcus*	Genus: *Propionibacterium*	None
An *et al*.[37] 2017 China	HIV+SD+ 13 HIV+SD - 16	HIV-SD+ 24 HIV-SD- 16		Scrub Bacterial culture (Only Staphylococcus studied)	Nasolabial fold Forearm	Genus: *Staphylococcus* HIV+, SD+ > HIV-, SD+ >	Genus: *Staphylococcus* HIV+, SD+ > HIV-, SD+ >	Genus: *Staphylococcus epidermidis* 80% *Staphylococcus aureus* 10% *Staphylococcus haemolyticus* 10% HIV+, SD- > HIV-, SD-
Tamer *et al*.[38] 2018 Turkey	SD 51	Control 50		Swab Bacterial culture	Frontal scalp	Genus: *Staphylococcus aureus* 49%	None	Genus: *Staphylococcus aureus* 20%
Puviani *et al*.[39] 2019 Italy	75			Swab Bacterial DNA	Glabella Nose-cheek furrow Mandibular rim	Genus: *Staphylococcus*	Genus: *Propionibacterium*	None
Park *et al*.[40]* 2017 Korea	SD 29	Dandruff 28	Control 45	Uncertain, next-generation sequencing	Scalp	Genus: *Staphylococcus*	None	Genus: *Propionibacterium*

*Only *Propionibacterium* and *Staphylococcus* were studied. SD+: Patients with seborrheic dermatitis, SD-: Patients without seborrheic dermatitis, HIV+: Patients with human immunodeficiency virus infection, HIV-: Patients without human immunodeficiency virus infection

the diversity measurement analysis in our study was that the richness and diversity values of the non-lesional groups lay between those of the lesional and control groups in both psoriasis and seborrheic dermatitis. This may indicate that non-lesional regions have the potential to turn into lesions in the future, though this idea requires further investigation.

Previous studies conducted to reveal the microbiota composition of healthy human skin have shown *Actinobacteria*, *Proteobacteria*, *Firmicutes* and *Bacteroidetes* to be the most dominant phyla.[7,8] The diversity and stability of microbiota change across different sites, and in some studies, *Actinobacteria* and *Firmicutes* have been determined to be the most abundant phyla on the scalp.[41,42] In our study, the healthy individuals' scalp microbiota were dominated by *Actinobacteria*, *Proteobacteria* and *Firmicutes*, as reported in another study.[43] When the microbiota compositions were examined in both disease groups at phylum level, similar to the overlapping differences detected in the alpha and beta diversity analyses, in both psoriasis vulgaris and seborrheic dermatitis groups, the abundance of *Actinobacteria* drastically decreased, and the ratios of *Firmicutes* and *Proteobacteria* increased, but the change in *Proteobacteria* was not statistically significant. The shift seen in *Actinobacteria* and *Proteobacteria* was lower in non-lesional sites compared to lesions, although this difference was not statistically significant. The phyla found in other studies were similar to those in our study, although some did not evaluate the scalp microbiota in non-lesional areas, or controls.[35,36,44] In parallel with the findings in alpha and beta diversity, we did not determine a significant difference between psoriasis vulgaris and seborrheic dermatitis.

The most dominant genus in the healthy hairy scalp microbiota is *Propionibacterium*, which was found in lower abundance in lesions of both psoriasis vulgaris and seborrheic dermatitis in our study, but no difference was detected between psoriasis vulgaris and seborrheic dermatitis. *Propionibacterium* plays a role in defense against pathogen invaders through the production of some molecules such as short-chain fatty acids and thiopeptides, showing an inhibitory effect against them. The decreased abundance of this bacterium in both disease groups in our study is consistent with the findings reported in several skin disorders, including dandruff, psoriasis and atopic dermatitis.[33,42,45,46] In contrast to the decreases in *Propionibacterium*, increased abundances of *Staphylococcus* and *Streptococcus* were found in the lesional skin samples of both disease groups, and to a similar extent. The increase in *Staphylococcus* and differences in *Propionibacterium* have been found to be associated with seborrheic dermatitis in other studies as well.[38,47] It has been suggested that these bacteria cause seborrheic dermatitis by providing nourishment for *Malassezia* through hydrolyzing sebum.[47] Conversely, the increase in the number of *Malassezia* in seborrheic dermatitis causes the increase of these lipophilic bacteria.[6] Similarly, in a study comparing patients with seborrheic dermatitis, those with dandruff and healthy individuals, it was reported that *Staphylococcus* was higher in

patients with seborrheic dermatitis than in the control group.[41] In another study, comparisons before and after treatment showed that *Staphylococcus* was high pre-treatment while *Propionibacterium* increased post-treatment in seborrheic dermatitis.[39] In yet another study of seborrheic dermatitis cases with ala nasi involvement, *Acinetobacter, Staphylococcus, Streptococcus, Corynebacterium* and *Propionibacterium* were detected in both lesional and non-lesional regions. Still, *Staphylococcus*, *Streptococcus,* and *Acinetobacter* were found to increase only in lesions, and *Propionibacterium* were detected more in non-lesional regions.[36] We did not find a statistically significant difference in bacteria between lesional and non-lesional areas in the two diseases. *Nocardioides* and *Anaerococcus* were found to be increased in psoriasis vulgaris lesions. *Anaerococcus* was previously found to be increased in skin samples but not the scalp of psoriasis vulgaris lesions. After narrow-band UVB treatment, there was a greater decrease in *Anaerococcus* in patients that responded to this treatment compared to the non-responsive group.[48]

In this study, *Finegoldia* was found to be increased in psoriasis vulgaris in comparison to the control group, but its abundance was very low. As an only described species of *Finegoldia*, *Finegoldia magna* is a part of the healthy human microbiota but also defined as an opportunistic pathogen.[49] We were unable to find any information explaining the role of this species in psoriasis vulgaris. A similar difference was seen for the genus *Mycobacterium*, another potential source of infection, which has been reported to cause infection on the scalp after skin grafting.[50] In the current study, the levels of *Haemophilus* were also increased in the psoriasis vulgaris group compared to the controls, but similar to *Finegoldia*, its load was very low. Several members of this genus are pathogenic and associated with several human infections.[51] They may not be specific to psoriasis vulgaris and potentially emerge due to the lost integrity of the skin.

Aquabacterium and *Enhydrobacter* were two genera detected in increased abundances in lesional seborrheic dermatitis areas compared to the healthy skin in controls. A genus identified in drinking water, we did not find any report indicating *Aquabacterium* as a member of the human skin microbiota. *Enhydrobacter* is a part of the human skin microbiota and has been reported to be found in very low abundance in hair roots.[52,53] The abundance of this genus has been reported to be increased in the skin samples of individuals in response to stress. There is as yet no pathogenicity potential ascribed to this genus.[54]

Finally, *Mycobacterium*, *Ezakiella* and *Peptoniphilus* were three genera detected only in the psoriasis vulgaris group, albeit at low concentrations. Only one study in the literature showed *Peptoniphilus* in psoriasis lesions, which was reported to decrease after narrow-band UVB treatment.[48]

Limitations

The small sample size is a limitation of the study. Studies comparing the two diseases with larger sample sizes are necessary. Higher taxonomic resolution down to species level is necessary and can be achieved by third-generation sequencing technologies, which can produce longer reads, or shotgun metagenomics sequencing that would allow for the examination of not only bacteria but also yeasts such as *Malassezi*a. Other omics analyses may also be beneficial to reveal the effect of dysbiosis on the host immune system.

Conclusion

Evaluated together, the analyses indicate the presence of greater diversity in the scalp microbiota in both psoriasis vulgaris and seborrheic dermatitis compared to healthy skin, suggesting the loss of mechanisms against foreign invaders. The results of the non-lesional samples in both diseases lay between those obtained from the control and lesional groups. Psoriasis vulgaris and seborrheic dermatitis were observed to share very similar microbiota compositions and diversity. The bacterial similarities between psoriasis vulgaris and seborrheic dermatitis suggest that these differences are a result rather than a cause of the respective diseases, and the lack of significant differences between the lesional and non-lesional psoriasis vulgaris samples, unlike in seborrheic dermatitis patients, indicates that psoriasis vulgaris is more severe than seborrheic dermatitis. However, more detailed studies should be planned to further investigate the differences in the microbiota integrated into the host's skin differences.

Declaration of patient consent

The authors certify that they have obtained all appropriate patient consent.

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Conflicts of interest

There are no conflicts of interest.

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