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RESEARCH PAPER

Malassezia and *Staphylococcus* dominate scalp microbiome for seborrheic dermatitis

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Abstract

Seborrheic dermatitis (SD) is a common disease of the human scalp that causes physical damage and psychological problems for patients. Studies have indicated that dysbiosis of the scalp microbiome results in SD. However, the specific fungal and bacterial microbiome changes related to SD remain elusive. To further investigate the fungal and bacterial microbiome changes associated with SD, we recruited 57 SD patients and 53 healthy individuals and explored their scalp microbiomes using next generation sequencing and the QIIME and LEfSe bioinformatics tools. Skin pH, sebum secretion, hydration, and trans-epidermal water loss (TWEL) were also measured at the scalp. We found no statistically significant differences between the normal and lesion sites in SD patients with different subtypes of dandruff and erythema. However, the fungal and bacterial microbiome could differentiate SD patients from healthy controls. The presence of *Malassezia* and *Aspergillus* was both found to be potential fungal biomarkers for SD, while *Staphylococcus* and *Pseudomonas* were found to be potential bacterial biomarkers. The fungal and bacterial microbiome were divided into three clusters through co-abundance analysis and their correlations with host factors indicated the interactions and potential cooperation and resistance between microbe communities and host. Our research showed the skin microbe dysbiosis of SD and highlighted specific microorganisms that may serve as potential biomarkers of SD. The etiology of SD is multi-pathogenetic-dependent on the linkage of several microbes with host. Scalp microbiome homeostasis could be a promising new target in the risk assessment, prevention, and treatment of SD disease.

Keywords Seborrheic dermatitis · Scalp microbiome · Dysbiosis · Biomarker · *Malassezia* · *Staphylococcus*

Introduction

Scalp seborrheic dermatitis (SD) causes erythema, dandruff, greasy hair, affecting the appearance, and self-esteem of patients. Moreover, it causes itchy skin and inflammation, and even hair losing. Therefore, SD has significant negative effects on patients' quality of life [1]. The prevalence of scalp SD in young adults was reported to be 3–5% [2] and up to 14.3% in the middle-aged and elderly population. Dandruff and erythema are the two main symptoms of scalp SD. Dandruff presents as visible white or yellow flakes and, as such, negatively influences the appearance of a person. In addition, dandruff often presents with itchy scalp, which causes discomfort to patients. Erythema is redness of the skin and can be accompanied by itching, tightness, or prickling sensations. Patients with dandruff often have erythema concomitantly. In addition, the prevalence of dandruff without erythema is nearly 50% in adults [3]. As SD of scalp causes physical damage and psychological problems for patients, it is important to research the mechanism of the disease. However, the pathophysiological processes of SD have not been thoroughly studied.

There are several theories regarding the pathogenesis of SD, including bacterial and fungal colonization of the scalp, imbalanced sebaceous gland activity, and individual

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susceptibility [4] scalp microbiota research has indicated that dysbiosis of the most common microbiota results in dandruff and/or SD [5, 6]. Recently, more and more researches on the pathophysiological theory of SD have been reported. However, the results of these researches are sometime conflicting. A previous study reported that while Basidiomycota was the most common phylum on scalps with dandruff, Ascomycota was most common in healthy scalps [7]. *Malassezia* is a genus of Basidiomycota, which are commensals of the skin that can occasionally provoke inflammatory reactions [8]. The clinical differences between dandruff and erythema are obscure. However, it remains unclear whether there is difference between the microbiota of the erythematous and the dandruff types of SD.

Scalp diseases are influenced by stratum corneum integrity, immune response, and neurogenic factors [1, 9, 10]. Soares et al. [11] suggested that systemic alterations of host conditions, such as Trans-epidermal Water Loss (TEWL), sebum production, and pH could alter the microbiota composition, and thereby cause dandruff. For example, sebum production is required to support growth of *Malassezia*. Changes in the skin pH can create a favorable environment for the growth of *Staphylococcus aureus* and exert an influence on the activity of the enzymatic process of lipid metabolism in the stratum corneum, possibly contributing to the impairment of skin barrier [12, 13]. The correlation between scalp physiological factors and microbiota has also not been closely assessed. Thus, we processed the analysis of a network of host factors and microbiota to find out whether there is a potential interaction between them.

Considering the majority of the studies cited above had very few subjects, and there has been no study on the microbiota of lesion and non-lesion areas of the scalp in SD patients, as well as the microbiota correlations suggested above with dandruff and erythema, we set-up this study. We had 110 volunteers who are residents in Beijing, China, and detected the scalp samples of lesion and non-lesion areas took from each volunteer in dandruff, erythema, and control group to investigate microbiota differentiation and potential biomarkers for SD and healthy scalps. To the volunteers were subsequently given suggestions for the treatment of the disease in the future.

Materials and methods

Subjects recruitment and classification

Volunteers that met the following five criteria were recruited into our study: (1) literate; (2) healthy (no any other diseases discovered including hypertension, diabetes and other chronic diseases, excluding SD of scalp); (3) 30–58 years of age; (4) resided in Beijing, China for over 5 years; and (5) of the Chinese Han population. Volunteers were excluded if they had: (1) concomitant skin diseases on the scalp; (2) significant scalp scar(s); (3) history of skin cancer; (4) history of an allergic reaction to shampoos, perfumes, or topically applied hair care products; (5) taken oral antibiotics, immunosuppressant, chronic anti-inflammatory, or chronic antihistamine drugs within 4 weeks prior to baseline measurements; (6) used anti-dandruff, anti-psoriatic, anti-seborrheic dermatitis shampoos, and/or therapeutic products within 2 weeks prior to baseline measurements; and (7) a significant medical condition or were on chronic medication.

According to the Declaration of Helsinki, all of the volunteers have been fully explained the procedure and purpose of the study, and signed an informed consent, prior to participation in this study. A total of 110 subjects consented to participate. Each subject was assessed by a dermatologist and classified into three groups—healthy group (53 volunteers), dandruff group (28 volunteers) and erythema group (29 volunteers), the dandruff group and erythema group together make-up the SD group. The age of the total subjects, healthy group, dandruff group, and erythema group were 43.84 ± 8.90, 44.34 ± 8.36, 41.61 ± 9.16, and 45.07 ± 9.21 years, respectively. The proportion of male subjects among the total subjects, healthy group, dandruff group, and erythema group are 46.36% (51/110), 47.17% (25/53), 46.43% (13/28), and 44.83% (13/29), respectively. There was no current or recent usage of antibiotics, immunosuppressant, anti-inflammatory, or antihistamine drugs for each one.

The assessment was based on photo, adherent scalp flaking score (ASFS) [14], active folliculitis, and erythema. The dandruff was assessed according to the ASFS. The scalp was divided into eight sections, and each section was assessed for the presence of dandruff flakes adhering to the scalp skin using a 0–10 (increment of 2 units) scale (0—no scaling, 2—slight scaling, 4—some scaling, 6—moderate scaling, 8—heavy scaling, 10—very heavy scaling). The final ASFS was the sum of the grades for all eight scalp sections, resulting in a scale ranging from 0 to 80 units. To evaluate the erythema, the scalp was divided into eight sections, and each section was assessed for the presence of erythema using a 0, 1 scale (0 = absence of erythema, 1 = presence of erythema). The final score of erythema scale was the sum of the grades for all eight scalp sections, resulting in a scale ranging from 0 to 8 units. The groups were divided according to the final ASFS and erythema score (normal group: erythema score=0, ASFS < 24; dandruff group: erythema score ≤ 1, ASFS ≥ 24; erythema group: erythema score > 1, or, erythema score $= 1$ and ASFS < 24). The lesion site was defined as the scalp section with the highest ASFS or with the presence of erythema in dandruff group or erythema group, respectively. One healthy site and one lesion site from each participant had its microbiota sampled, sequenced, and

analyzed. As for control subjects, one healthy site and one replicate site were sampled. Our study and all experiments were implemented in accordance with the ethical principles and regulations.

Information collection and physiological measurement

We collected demographic information by general questionnaire, and sensitivity condition with respect to itching, tightness, burning, prickling sensation and pain by self-assessment of Sensitive Scalp Score [15]. Additionally, scalp physiological parameters were measured by instrument at mid vertex center and occipital promontory, mainly with site of lesion (flake region, erythema). Subjects had to use a non-dandruff, non-seborrheic dermatitis shampoo for 2 weeks prior to measurement, with the last shampoo performed 24 h before the measurement. They were rested for 15 min in an environmentally controlled room (temperature 25 °C, relative humidity 40%). TEWL and hydration of each site were examined three times by Vapometer (Delfin) and Corneometer CM 825 (Courage+Khazaka, Koln, Germany), respectively, and the average was used for analysis. The sebum was examined with a Sebumeter SM815 (Courage+Khazaka, Koln, Germany) once for 20 s. Moreover, the assessor measured pH by a Skin Ph meter PH900 (Courage+Khazaka, Koln, Germany), and took a picture of the scalp from two points by folliscope (Magnification: 30×).

Sampling, DNA extraction and polymerase chain reaction

We collected microbial samples at healthy scalp and lesion scalp, by swabbing for 30 s with Catch-all Sample Collections Swabs (QEC091H, Epicentre, Madison, USA). The swab samples were stored at − 20 °C until bacterial and fungal DNA extraction.

DNA was extracted from the microbial flora contained in the scalp samples using the Oral Swab DNA Rapid Extraction Kit (Centrifugal column type) (Beijing BioTeke) following the manufacturer's specifications.

All 64 individually processed human scalp swab gDNA extractions were PCR amplified. The 514F (GTGCCA GCMGCCGCGGTAA) upstream primers and 805R (GGA CTACHVGGGTWTCTAAT) downstream primers were used to amplify the 16S rRNA gene V4 region from the bacteria. Amplifications were performed using a step cycling protocol consisting of 94 °C for 2 min, 30 cycles of 94 °C for 30 s, 54 °C for 30 s, and 72 °C for 45 s. A condition of 72 °C for 5 min was used for the final elongation. Each 25 μL PCR reaction contained 0.5 μL of template DNA, 2.0 μL of dNTP mix (2.5 mmol/L; TaKaRa), 2.5 μL of TaKaRa 10× Ex Taq buffer (Mg^{2+} free), 1.5 μL of Mg^{2+} (25 mmol/L), 0.25 μL of TaKaRa Ex Taq DNA polymerase (2.5 units), 0.5 μL of 10 μmol/L bar code primer 514F, 0.5 μL of 10 μmol/L primer 805R, and 17.25 μL of double-distilled water. Likewise, the ITS1-F (CTTGGTCATTTAGAGGAAGTAA) upstream primers and ITS1-R (GCTGCGTTCTTCATC GAT GC) downstream primers were used to amplify the ITS1 region from fungi [16]. Amplifications were performed using a step cycling protocol consisting of 94 °C for 15 min, five cycles of 94 °C for 30 s, 50 °C for 30 s, and 72 °C for 1 min. Then, 35 cycles of 95 °C for 30 s, 65 °C for 30 s, and 72 °C for 1 min were performed. Condition of 72 °C for 10 min was used for the final elongation. Each 20 μL PCR reaction contained 10 ml of Maxima Hot Start PCR Master Mix (Thermo, USA), 2 μL of template DNA, 0.5 μL of ITS upstream primers, 0.5 μL of ITS downstream primers, and 7 μL of double-distilled water.

Double-end sequencing and microbiome data analysis

According to the manufacturer's protocol, all PCR amplicons were sequenced using Illumina Hiseq (PE 250). Sequences were analyzed using QIIME version 1.9.1. The original double-end data was spliced with SeqPrep. Low quality sequence was filtered with an acceptable minimum quality score of 20. Sequences were divided into each sample by barcode, and then barcode and primers were removed. The sequence number of each of the groups was standardized into 1350 and 3230 after the completion of OTU from bacteria and fungi. We used QIIME for analyses of α-diversity and β-diversity, and linear discriminant analysis effect size (Lefse) for biomarker discovery. Statistical analysis of the clinical data and graphing were implemented using R (v3.3.2). The Kruskal–Wallis test was used to determine significance among multi-groups and Wilcoxon rank sum test was used to determine significance between two groups. The box plots of α-diversity and the content graph of biomarkers in positive or negative position were accomplished using ggplot2. From the phylum to genus level, bacteria with an average value greater than 0.01 were shown, while the phylum to genus level of fungi were shown with average value greater than 0.001.

Results

Scalp skin microbiomes are generally similar in lesion and healthy sites

The recent investigations have greatly extended our understanding of SD. However, the microbial and fungal microbiome statue of different subtypes of SD patients and sampling sites remain elusive. In this study, scalp swab samples were

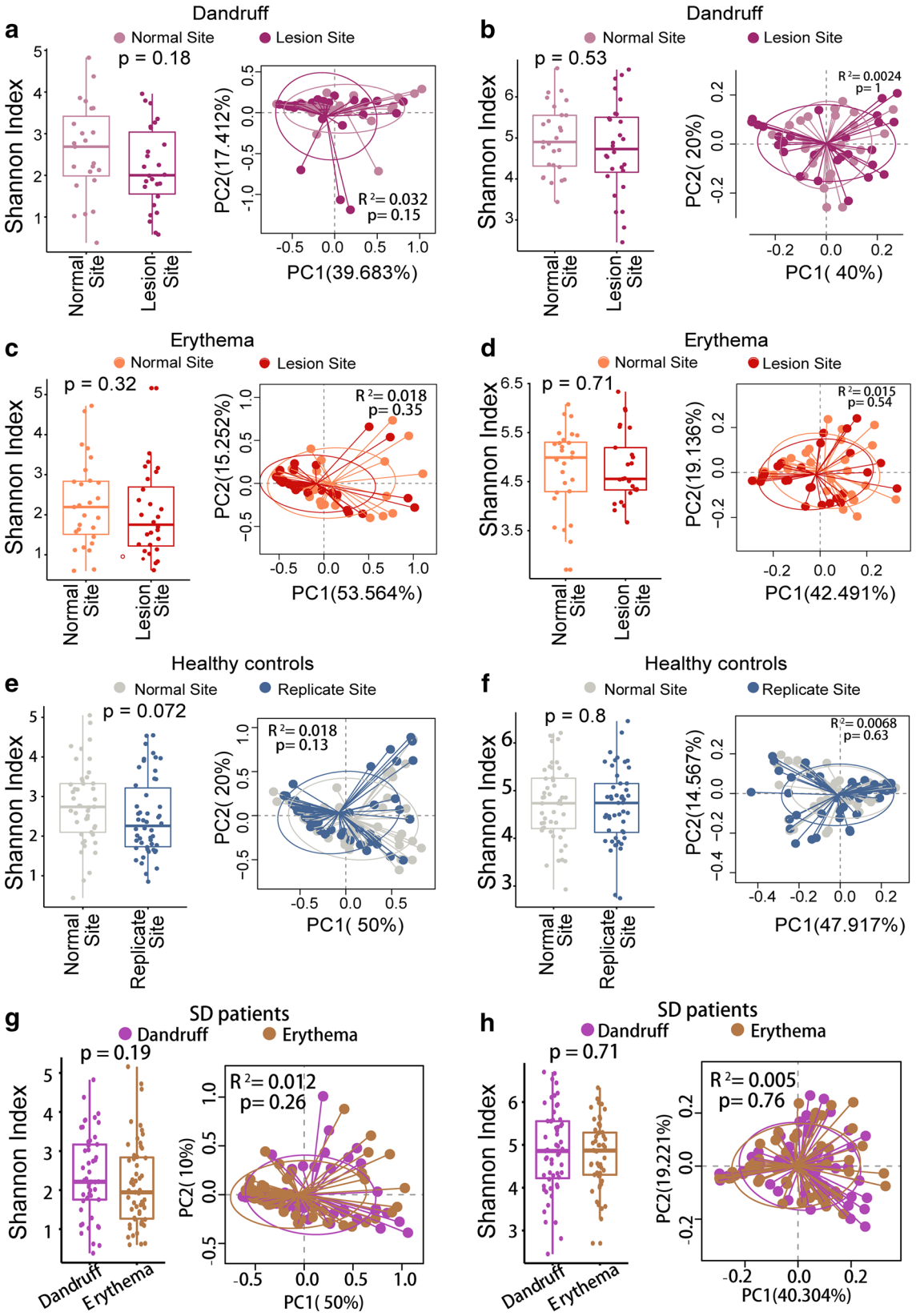

Fig. 1 **a**, **c**, **e**, **g** Fungal diversity comparison between the normal site and lesion site from the same patients. **b**, **d**, **f**, **h** Bacterial diversity comparison among the normal site and lesion site. **a**, **b** Dandruff group. **c**, **d** Erythema group. **e**, **f** Healthy group. **g**, **h** SD group

taken from participants before administration of antibiotics or any medical treatment. Shannon index and principal coordinate analysis (PCoA) based on the unweighted UniFrac distance were used to evaluate α-diversity and β-diversity respectively (Fig. 1). While the Shannon index describes both the abundance and evenness of each sample, PCoA compares the composition difference between groups. Our results showed that the composition and diversity of fungi (Fig. 1a, c, e) and bacteria (Fig. 1b, d, f) were not significantly altered in lesion sites as compared to healthy sites in neither dandruff nor erythema group.

SD patients suffer from both dandruff and erythema. Diagnostic criteria differentiating the two types of seborrheic dermatitis remain vague. According to our analysis, there were no significant changes in both α-diversity and β-diversity between dandruff and erythema in fungi (Fig. 1g) or in bacteria (Fig. 1h).

We also found that the healthy controls and SD patient groups, and the subtype groups of normal and lesion sites of Dandruff and Erythema, shared most of the predominant microbe. The most abundant fungal phyla were Basidiomycota and Ascomycota, which together contribute over 95% of the fungal microbiome (Fig. S1a). The predominant eight fungal genera which constituted over 75% of the total fungal sequences were *Malassezia, Aspergillus, Exophiala, Aureobacsidium, Phaeoacremonium, Lecanicillium, Cyberlindnera*, and *Debaryomyces* (Fig. S1b). As for bacteria, Proteobacteria, Firmicutes, Bacteroidetes, Actinobacteria, and Cyanobacteria consisted over 95% of the phylum level (Fig. S1c). The top 8 predominant genera were *Staphylococcus, Sediminibacterium, Corynebacterium, Pseudomonas, Phyllobacterium, Bardyrhizobium, Sphingomonas,* and *Bosea*, which constituted over 50% of the total bacterial sequences (Fig. S1d).

Classification models based on the fungal microbiome are more effective than bacterial models to differentiate SD patients from healthy controls

To investigate the bacterial and fungal microbiome differences between seborrheic dermatitis patients and healthy controls, diversity analysis was performed. The Shannon index of fungal microbiota in the seborrheic dermatitis group was statistically significantly lower than the control group, indicating that the healthy group harbored greater fungal diversity than the seborrheic dermatitis group. While there were overlaps between fungal microbiomes of the SD patients and those of the healthy controls in the PCoA, PERMANOVA showed statistical power in distinguishing the two groups. Also, we constructed a random forest model using the fungal microbiota to distinguish the two groups and the AUC of fungal microbiota model was 73.82%, indicating fare discrimination ability (Fig. 2a), as an AUC above 80% is generally considered well classification accuracy.

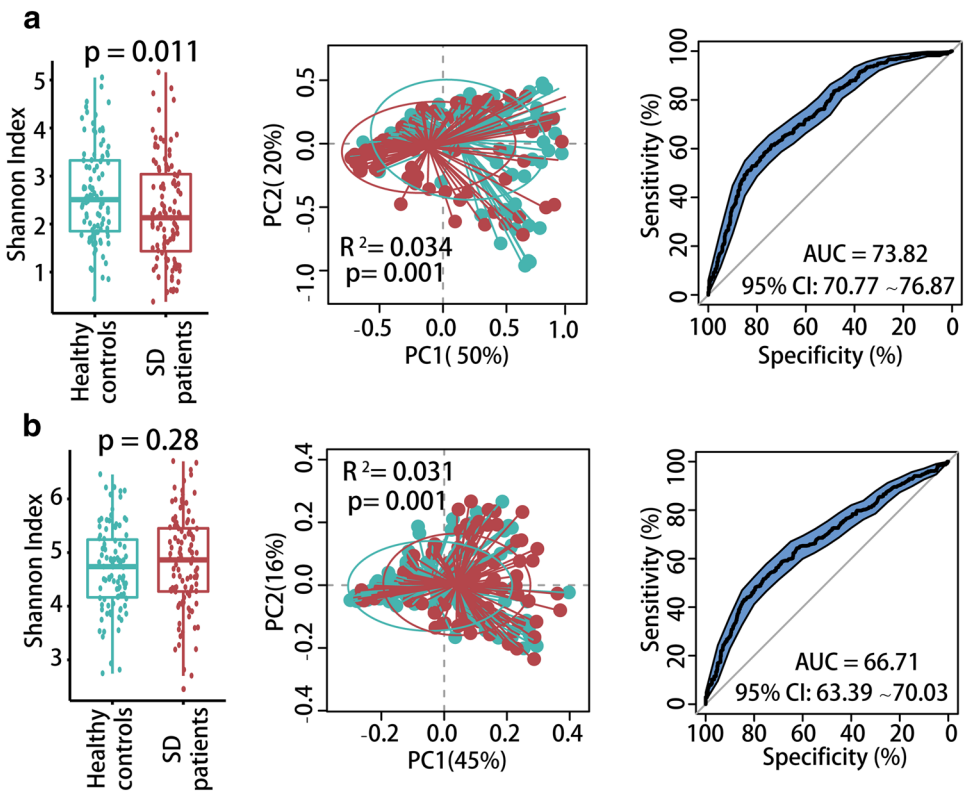

Fig. 2 Shannon index (left), PCoA based on Unweighted Unifrac distance (middle) and random forest model Receiver operating characteristic (ROC) curve (right) of fungi (**a**) and bacteria (**b**) between the Healthy controls and SD patient groups

The above analyses were also performed based on the bacterial microbiota. Although the Shannon index showed no significant shift between the healthy controls and SD patient groups, the two groups could be distinguished by PCoA (PERMANOVA, $p = 0.001$). The AUC based on the bacterial microbiota model was 66.71%, indicating weaker classification efficiency than the fungal model (Fig. 2b).

Taxonomic biomarkers that are distinct between the SD patients and healthy controls

To further identify which fungal and bacterial taxa were significantly different between the two groups, LEfSe (Linear Discriminant Analysis Effect Size, LEfSe) [17] analysis was applied. Based on the LDA effect size, LEfSe analysis showed that two fungal genera (*Malassezia* and *Mycosphaerella*) and two bacterial genera (*Staphylococcus* and *Brevibacterium*) were enriched in the SD patient group, while five fungal genera (*Aspergillus*, *Ganoderma*, *Exidia*, *Pilatoporus,* and *Engyodontium*) and five bacterial genera (*Pseudomonas*, *Hyphomicrobium*, *Proteus*, *Devosia,* and *Bacteroides*) were enriched in healthy controls (Figs. 3a, 4a).

Among these genera, *Malassezia* contributed 43% and 54% fungal abundance in healthy controls and SD patient group, respectively; *Aspergillus* contributed 19% and 12% fungal abundance in healthy controls and SD patient group, respectively. While *Staphylococcus* contributed 16% and 22% bacterial abundance in healthy controls and SD patient group, respectively; *Pseudomonas* contributed 10% and 7% bacterial abundance in healthy controls and SD patient group, respectively (Figs. 3b–e, 4b–e). In this study, there was no significant difference between the relative abundance

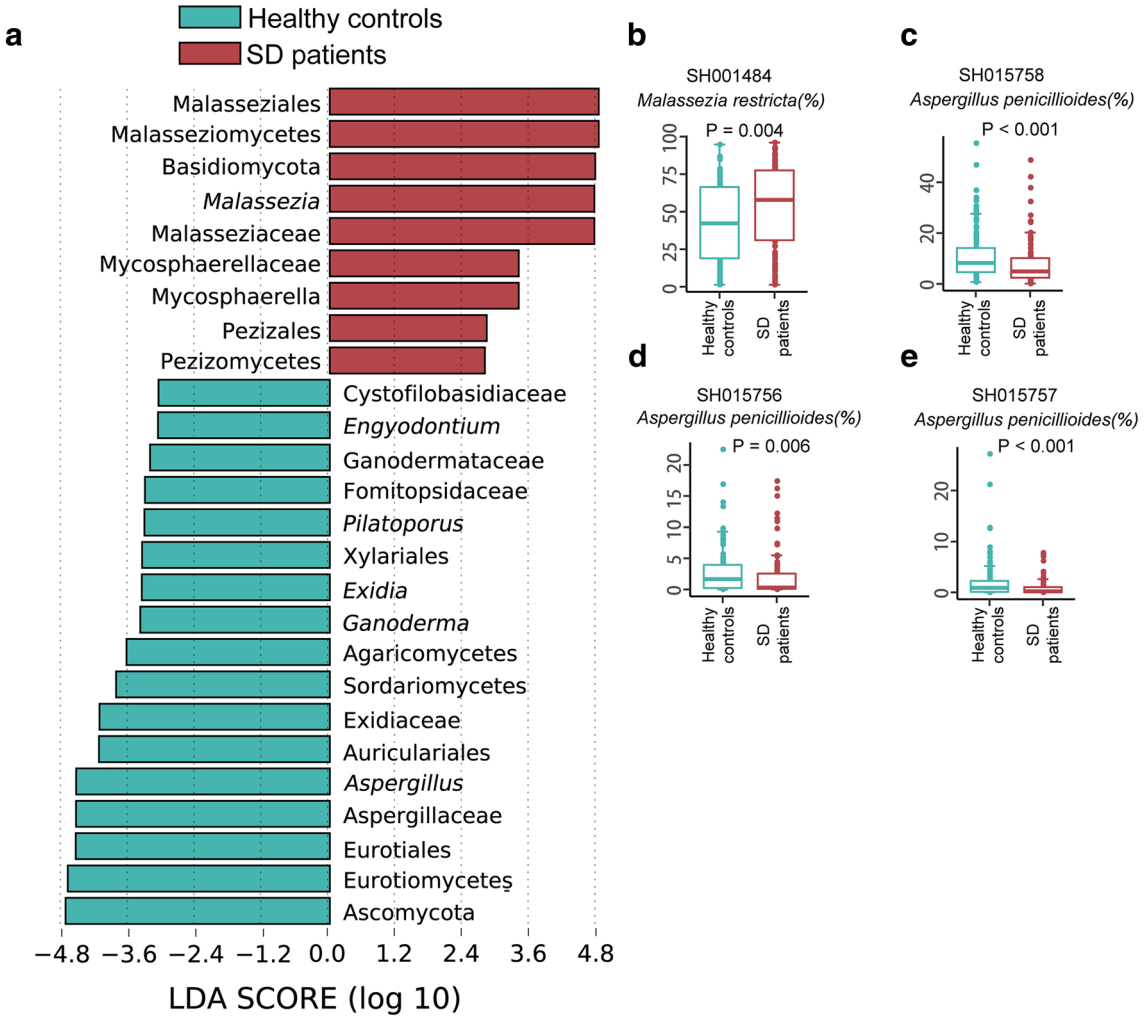

Fig. 3 **a** LEfSe results showing significantly different fungal taxa between the healthy controls and SD patient groups. SH001484 *Malassezia restritica* (**b**), SH015758 *Aspergillus penicillioides* (**c**), SH015756 *Aspergillus penicillioides* (**d**), SH015757 *Aspergillus penicillioides* (**e**) in the SD patients (SD) and control (N) group

of *Propionibacterium acnes* in the SD patient group (0.7%) and healthy controls (0.6%).

At the operational taxonomic unit (OTU) level, because *Malassezia*, *Staphylococcus* and *Pseudomonas* were dominated by a single OTU, their OTUs showed consistent trend with these genera (Figs. 3b, 4b–e). Meanwhile, *Aspergillus* consisted of eight OTUs, which indicated that *Aspergillus* has a higher phylogenetic diversity than the other biomarker genera (Fig. 3c–e).

Correlation network of microbiota and host factors

Furthermore, we conducted co-abundance group network analysis to study the relationship between fungi and bacteria. Genera which have higher than 1% mean abundance and were significantly statistically different between the SD and healthy groups were selected for the analysis.

The co-abundance analysis resulted in three major clusters. The first cluster consisted of some genera increased in seborrheic dermatitis group. The second cluster consisted of genera decreased in seborrheic dermatitis group. In addition, *Aspergillus* alone was the third cluster. It is noteworthy that most of the microbes in the first cluster were negatively correlated with microbes in the second cluster, implying the correlation and interactions of these two clusters. Aspergillus were not clustered with any other genera which might be due to its sporadic abundance among different OTUs (Fig. 5).

We further expanded the co-occurrence analysis to include OTUs and host physiological traits. There were also three major clusters observed, mainly dominated by

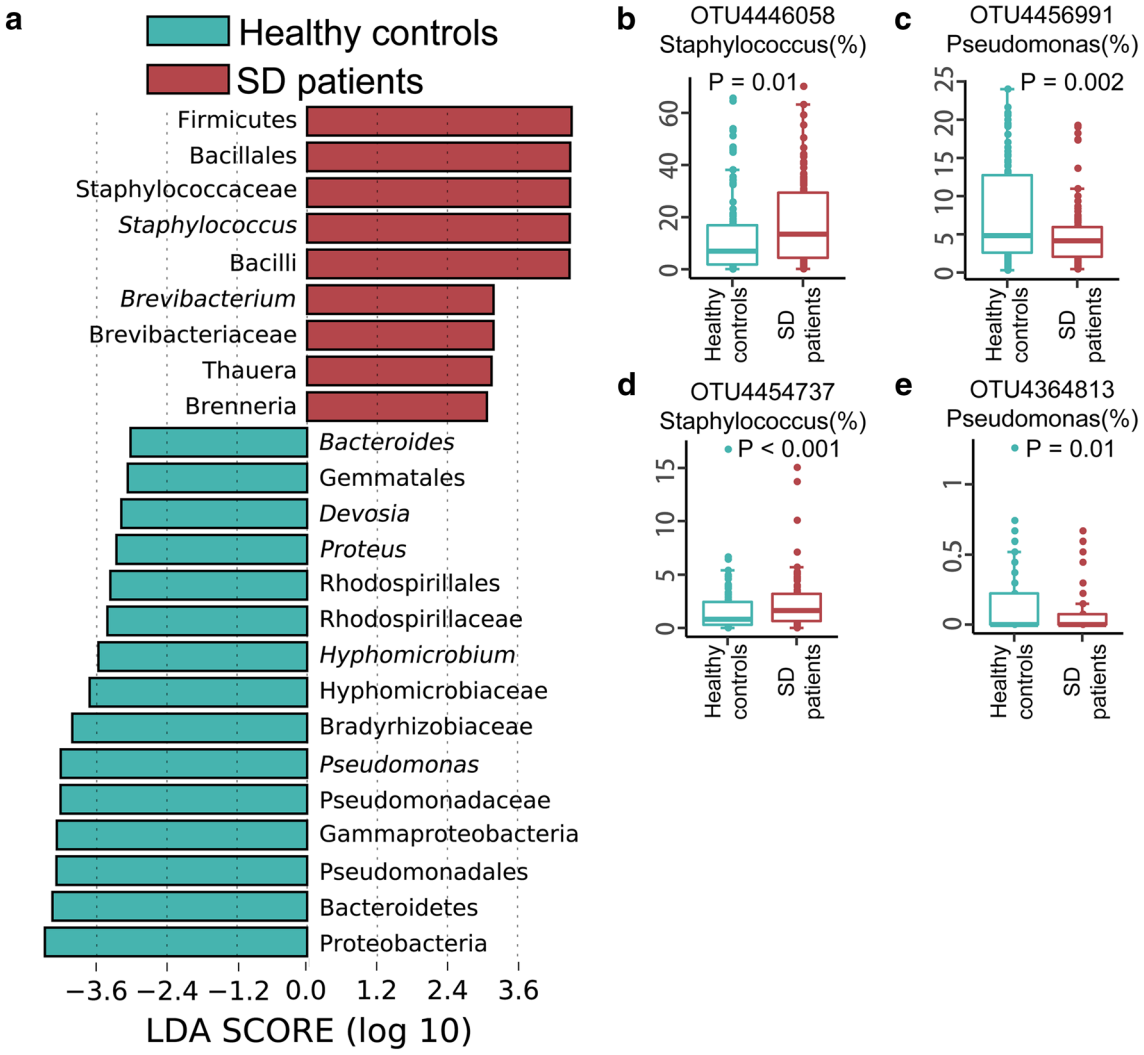

Fig. 4 **a** LEfSe results showing significantly different bacterial taxa between the healthy controls and SD patient groups. OTU4446058 *Staphylococcus* (**b**), OTU4456991 *Pseudomonas* (**c**), OTU4454737 *Staphylococcus* (**d**), OTU4364813 *Pseudomonas* (**e**) in the SD patients (SD) and health(N) group

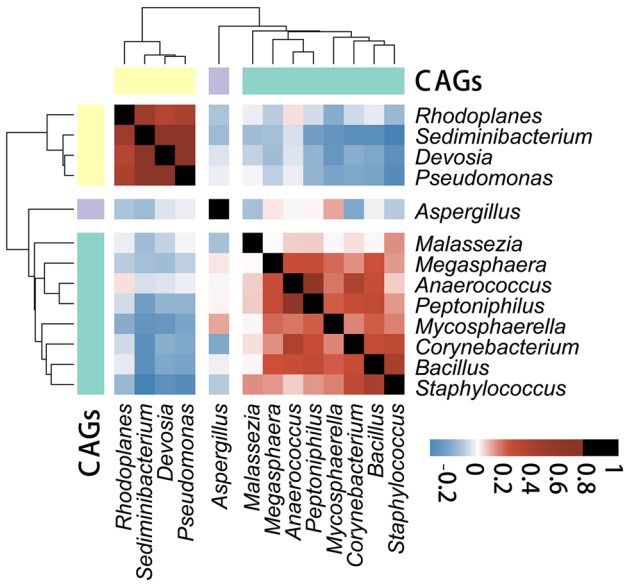

Fig. 5 Co-abundance correlation analysis (based on the Spearman's correlation, $p < 0.05$) of the statistically significant genera between SD patients. Red blocks represent positive correlation between microorganisms and blue blocks represent negative correlation. Yellow, purple, and light green represent three different CAGs (color figure online)

Malassezia restrita and *Staphylococcus* (top-left), *Aspergillus penicilloides* and *Sediminibacterium* (top-right), and *Corynebacterium* (bottom) respectively. The clusters on the top-left and top-right consisted of OTUs enriched and decreased in SD respectively. These two clusters were mainly negative correlations. The third cluster consists of some low-abundance OTUs enriched in SD and positively correlated with the top-left cluster while not correlated with the top-right cluster.

As for the interaction with host, sebum was positively correlated with SH001484.07FU_AY743636_refs (*Malassezia restrita*) and negatively correlated with SH015758.07FU_FR727125_reps (*Aspergillus penicilloides*), while pH was positively correlated with *Staphylococcus*, hydration was positively correlated with *Devosia* and *Sediminibacterium*, TEWL was positively correlated with *Rhodoplanes* (Table 1; Fig. 6).

Discussion

Seborrheic dermatitis is one of the most common scalp disorders. Clinical practice and recent studies into the scalp microbiome have focused mainly on the classic fungus Malassezia. However, few studies have investigated both the fungal and bacterial scalp microbiome and its relationship with SD. One of the key findings of this study is that we have identified two sets of candidate microbes that were significantly related to SD. Of note is that many of these genera are the most important scalp pathogens, including *Malassezia*, *Staphylococcus, Aspergillus*, *Pseudomonas*, etc. Fungal and/or bacterial biomarkers can be developed as an alternative clinical evaluation for the success of SD treatment, complementing traditional measures that focus primarily on visible signs and symptoms [18, 19]. Our finding indicates that SD is a multi-pathogenic disease, caused by dysbiosis of both fungi and bacteria, rather than a mono-pathogenic disease.

In the present study, the fungal microbiome is significantly more different in SD compared with control samples than the bacterial microbiome, indicating more relevance for fungi in SD disease. Among these identified pathogens, over 99% abundance of genera *Malassezia* is from one OTU, SH001484.07FU_AY743636_refs_Malassezia.restrita, which is reported as one important potential pathogen. Interestingly, although other genera, such as *Staphylococcus*, *Aspergillus*, and *Pseudomonas* also compose several abundant OTUs, the shift tendency of these OTUs are mostly consistent within genera. This finding indicates that the relationship between particular diseases and pathogens are similar for microbes of the same genera.

Another key finding of this study is the potential interactions among fungi, bacteria and host. There are three major clusters dominated by *Malassezia*, *Aspergillus,* and *Corynebacterium*, respectively. Within each cluster, they

Table 1 Physiological data of different groups

Physiological factors	Normal group ($n = 53$)		Dandruff group ($n = 28$)		Erythema group ($n = 29$)	
	Front site	Rear site	Front site	Rear site	Front site	Rear site
TEWL (g/m^2 h)	17.76 ± 5.06[a]	17.57 ± 6.48	18.10 ± 4.89	17.53 ± 8.91	17.18 ± 4.26	17.18 ± 4.20
Hydration (CU)	5.35 ± 4.13	8.40 ± 8.13	3.04 ± 2.02	4.36 ± 5.04	4.62 ± 3.41	7.11 ± 8.12
Sebum (μg/cm^2)	128.43 ± 116.15	75.15 ± 62.58	156.86 ± 128.75	111.93 ± 109.88	196.97 ± 160.39	121.72 ± 117.37
pH	4.59 ± 0.46	4.47 ± 0.42	4.78 ± 0.48	4.71 ± 0.41	4.68 ± 0.36	4.57 ± 0.39

TEWL Trans-epidermal water loss

[a]The value is mean value ± standard deviation, as all of the data

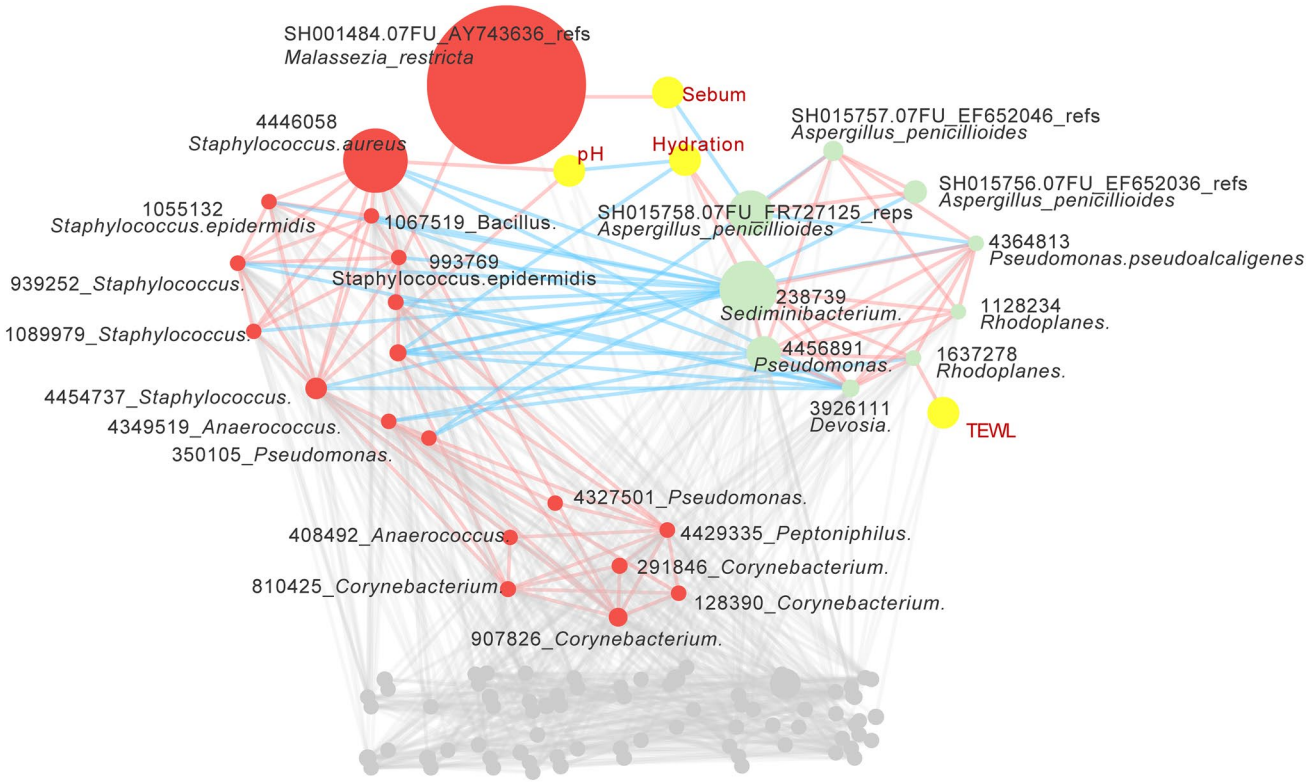

Fig. 6 Correlation networks of fungi, bacteria, and host factors. The larger relative abundance corresponded to the bigger size of the node. The orange lines represent positive relationships, the blue lines represent negative relationships and gray lines represent not significant correlation. Red dots represent OTUs enriched in SD group, green dots represent OTUs decreased in SD group, yellow dots represent host factors and gray dots represent not significant OTUs between Healthy controls and SD patient groups (color figure online)

are mostly positively correlated. However, the *Malassezia* cluster and *Aspergillus* cluster are negatively correlated, which is consistent with the correlations of these microbes and seborrheic dermatitis. In addition, we found sebum secretion correlates with two of the most abundant fungal OTUs from *Malassezia restricta* and *Aspergillus penicillioides*, respectively. Previous study revealed that *Malassezia restricta* are lipophilic fungi, whose lipases are responsible for the hydrolysis of triacylglycerols (the main component of human sebum) and finally lead to the release of unsaturated fatty acids [1, 10, 20, 21]. *Staphylococcus* thrive in an environment with higher pH value. *Staphylococcus* (*S. aureus*) products could cause exudate, which in turn would further increase the pH, continuing a vicious cycle. The microbiota influences the homeostatic environment of the scalp, which is consistent with the conjecture about the combination of fungal and bacterial colonies previously published. The beginning of this cycle could even be a precipitating factor or cause of SD [5]. However, scalp microbiomes might vary with different geographical regions [22], and thus multi-regional cohort studies are needed to verify the applicability of these biomarkers. Also, by comparing the samples from different lesions and SD clinical performances, no statistically significant changes are observed between different groups. This may be due to spreading of bacteria from scratching or combing.

In a whole, the findings of our study are consistent with those of previous studies. It has been demonstrated that *Malassezia* is the main causative pathogen in SD [23, 24]. Adherence of the *Malassezia* species to the scalp can impair the scalp barrier function of the stratum corneum through the release of unsaturated fatty acids [25]. The pathways related to general functions and genetic information and processing (sulfur relay system, proteasome pathway, cell cycle in yeast, and meiosis) showed a significant positive correlation with *M. restricta* and a positive correlation of these pathways was observed with dandruff-associated clinical parameters (dandruff score, itching and TEWL) [26]. It has also been reported that some metabolic products of tryptophan produced by *Malassezia*, such as indole derivatives, cause dandruff [27]. Tamer et al. revealed that *S. aureus* was the most common bacterial species isolated from SD patients [28]. A previous study suggested that bacterial microbiota such as *Staphylococcus* (*S. aureus*) might play a role in the development of seborrheic dermatitis by hydrolyzing sebum and providing nutrients for *Malassezia* [29]. *Staphylococcus* was

found positively correlated with dandruff scores, TEWL, and itching, while *Pseudomonas* showed a negative correlation with these parameters [26].

However, our study is just based on the observational data, future studies that define the causative relationship between biomarkers and seborrheic dermatitis will be required. In addition, the multi-faceted relationship between fungi, bacteria and host factors indicating cooperation and antagonism that contribute to SD are promising, and more studies on the subject are required.

In conclusion, our results showed that fungi and bacteria communities change in seborrheic dermatitis patients and revealed the potential interactions of fungi, bacteria, and host. This study implies that SD may be a dysbiosis-related disease rather than an infectious one. Scalp microbiome homeostasis could be a promising new target in the risk assessment, prevention, and treatment of SD disease.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Human and animal rights The authors declare that the research is ensuring compliance with ethical standards, and all of the human participants have been fully explained the procedure and purpose of the study, and signed an informed consent, while the research did not involve animals.

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