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CONFLICT OF INTERESTS

The authors have declared no conflicting interests.

AUTHORS' CONTRIBUTIONS

KFH designed the research. KHM analysed the data. PSL and BWC carried out the experiments. SHC drafted the manuscript.

Keywords
calcium influx, epidermal growth factor, keratinocyte differentiation

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REFERENCES

[1] M. A. Lowes, A. M. Bowcock, J. G. Krueger, *Nature* **2007**, *445*, 866.
[2] A. Menter, C. E. Griffiths, *Lancet* **2007**, *370*, 272.
[3] B. Canguilhem, A. Pradines, C. Baudouin, C. Boby, I. Lajoie-Mazenc, M. Charveron, G. Favre, *J. Biol. Chem.* **2005**, *280*, 43257.
[4] C. Meulenbroeks, H. van Weelden, C. Schwartz, D. Voehringer, F. A. Redegeld, V. P. Rutten, T. Willemse, A. J. Sijts, D. M. Zaiss, *J Invest Dermatol* **2015**, *135*, 222.
[5] G. K. Menon, S. Grayson, P. M. Elias, *J Invest Dermatol* **1985**, *84*, 508.
[6] A. Celli, S. Sanchez, M. Behne, T. Hazlett, E. Gratton, T. Mauro, *Biophys. J.* **2010**, *98*, 911.
[7] M. Muller, K. Esin, K. Hill, H. Beschmann, S. Rubant, C. M. Schempp, M. Gollasch, W. H. Boehncke, C. Harteneck, W. E. Muller, K. Leuner, *J. Biol. Chem.* **2008**, *283*, 33942.
[8] M. Moravcova, A. Libra, J. Dvorakova, A. Viskova, T. Muthny, V. Velebny, L. Kubala, *Interdiscip Toxicol* **2013**, *6*, 203.
[9] A. Gazel, T. Banno, R. Walsh, M. Blumenberg, *J. Biol. Chem.* **2006**, *281*, 20530.

SUPPORTING INFORMATION

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Data S1 Experimental design

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Collapse of human scalp microbiome network in dandruff and seborrhoeic dermatitis

Abstract

We investigate the relationship between scalp microbiota and dandruff/seborrhoeic dermatitis (D/SD), an unpleasant scalp disorder common in human populations. Bacterial and fungal community analyses on scalp of 102 Korean were performed by next-generation sequencing. Overall scalp microbiome composition significantly differed between normal and disease groups, and especially co-occurrence network of dominant members was breakdown in disease groups. These findings will provide novel insights into shifts of microbial community relevant to D/SD.

1 | BACKGROUND

Human scalp accommodates diverse bacteria and fungi that influence both healthy and diseased scalps. *Stahylococcus*, *Propionibactericum* and *Malassezia*, a common scalp commensal microorganism, are widely known as the cause of most scalp diseases in humans, including common dandruff and seborrhoeic dermatitis (D/SD).[1,2] However, controversy still remains about the microbial species responsible for scalp diseases. There were few comprehensive studies on scalp bacteria and on the complexity of normal, commensal communities of the

human scalp.[3,4] A hypothesis states that collapse of a balanced microbiome, such as a reduction in microbial diversity and overgrowth of some microbial species, may be involved in skin problems. D/SD is a common affliction characterized by visible flaking, itching, burning and pain, which affects almost half of the postpubertal population regardless of ethnicity and gender.[5,6]

In this work, we investigated the bacterial and fungal microbial communities on scalps associated with D/SD, using the Illumina MiSeq sequencing platform. The criteria to classify scalp samples into the three groups were as follows: (i) normal, adherent scalp flaking score (ASFS) <10; (ii) dandruff, ASFS ≥24; and (iii) SD, ASFS ≥24 with erythema.

2 | QUESTIONS ADDRESSED

We questioned (i) whether there was any difference between diseased scalps and normal scalps with respect to their microbial community structure and diversity and (ii) whether the bacterial and fungal network collapsed in diseased scalps compared to normal scalps.

3 | EXPERIMENTAL DESIGN

All methods are described in details in supplementary data (Data S1).

4 | RESULTS

To determine the composition of human scalp microbial communities of D (n=28) and SD (n=29) groups compared to normal (n=45) group

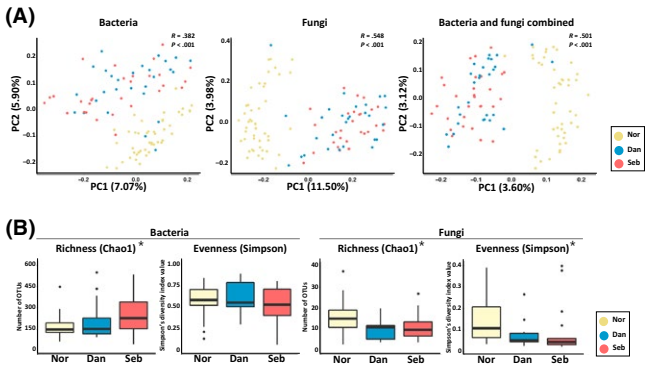

FIGURE 1 Bacterial community and fungal community are significantly divided into normal and disease groups (normal [Nor], dandruff [Dan], seborrhoeic [Seb]). (A) Principal coordinates analysis (PCoA) plots were based on normal and disease groups (normal=45, dandruff=28, seborrhoeic=29) of (left) bacterial PCoA by unweighted Unifrac distance (P<.001, analysis of similarities [ANOSIM]), (middle) fungal PCoA by Canberra distance (P<.001, ANOSIM) and (right) bacteria and fungi combined PCoA of bacterial OTUs and fungal species by Canberra (P<.001, ANOSIM). (B) Richness (chao1) and evenness (Simpson) of bacteria and fungi. Asterisk indicates (*) a significant statistical difference (P<.05, ANOVA)

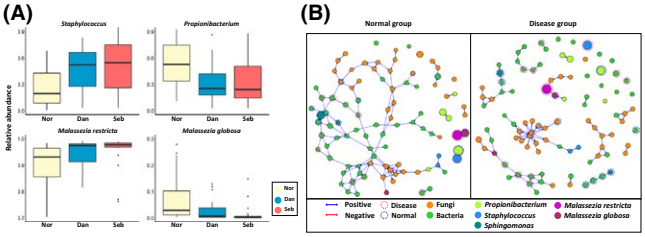

FIGURE 2 Relative abundance of major microbial genus and species found on the scalp and network of bacterial OTUs and fungal species of the normal and disease groups. (A) Relative abundance of bacterial genus *Staphylococcus* and *Propionibacterium*, fungal species *Malassezia restricta* and *Malassezia globose*. (B) Networks of the normal and disease group were constructed with bacterial 56 OTUs having average relative abundance over 0.05% and 39 fungi species having average relative abundance over 0.01%. Each node indicated 56 OTUs of bacteria or 39 species of fungi. The node size corresponded to relative abundance of each OTUs and fungal species, respectively. The red or blue dot circles outside of some nodes mean more relative abundance in the disease group (red dot circle) and more relative abundance in the normal group (blue dot circle), respectively. The OTUs and the fungal species were selected on the basis of Gini index top 30

in Korean population, bacterial and fungal communities of 102 human scalp samples were analysed by Illumina MiSeq (Table S1, Figure S1, Data S1). Principal coordinates analysis (PCoA) revealed appreciably distinct bacterial and fungal communities among normal and disease (D/SD) groups (Figure 1A). The disease status was the major factor used to separate bacterial (analysis of similarities [ANOSIM] R=.382; P=.001) and fungal communities (R=.548, P=.001) from normal groups (Table S2). The bacterial richness (Chao1) was significantly higher in disease groups than normal, whereas an inverse trend was observed in fungal richness (Figure 1B). Both bacterial evenness and fungal evenness (Simpson's evenness index) were decreased further in the dandruff and seborrhoeic groups than in the normal group (Figure 1B), which indicates an increase in the dominance of particular bacteria and fungi in the disease groups. Analysis of variance (ANOVA) found that the disease groups had higher level of active folliculitis and pH but a lower level of hydration (Table S3, Figure S4). Interestingly, symptomatic factors (prickling, itching, pain and burning) were significantly correlated only to the bacteria community composition, by ANOSIM test (Table S2). In the scalp microbial community of the volunteers with these symptoms, some candidate bacteria are considered to cause these symptoms, including *Hymenobacter* and *Deinococcus that* were identified (Table S3).

Relative abundances of two predominant bacteria, *Staphylococcus* and *Propionibacterium*,[7] and of predominant fungi, *Malassezia restricta* and *Malassezia globosa*, behave inversely on the scalp based on disease conditions (Figure 2A). We also conducted Random Forest supervised learning models to investigate the most differentiating taxa among three groups, determined by its value of mean decrease in Gini coefficient (Figures S2,S3). *Bacteroides* (dandruff), *Propionibacterium* (the most close to *Propionibacterium avidum* with 98% BLAST identity; SD) and *Chryseobacterium* (SD) revealed by Random Forest analysis showed

increases in diseases groups, whereas the genus *Rhizobium*, *Gordonia*, and *Sphingomonas* showed increase in normal group. In network analysis, the disease groups showed lower connectivity and less complex bacterial and fungal network than the normal group (Figure 2B). We calculated the network density (linkage complexity), which is the ratio of the number of edge, and the value of normal (0.026) was higher than disease group (0.019), suggesting that the healthy scalp microbiota were more stable connections among dominant genus and species than disease groups.

5 | CONCLUSIONS

We found that bacterial and fungal communities were different between disease (D/SD) and normal groups. Through statistical analysis, we concluded that these differences might be affected by the disease condition (Table S2) of the human scalp. Interestingly, both bacterial and fungal communities appeared to be associated with the human scalp disorder; however, we suggest that physical pain in scalp diseases is caused by bacterial community. This may also indicate that bacteria, such as *Bacteroides*, *Propionibacterium* and *Chryseobacterium*, have a significant effect on the symptoms of the scalp disease. It was observed that *Staphylococcus* sp. and *M. restricta* were associated with a higher incidence of scalp disease, whereas *Propionibacterium* sp. and *M. globosa* were associated with normal scalp.[7,8]

Staphylococcus sp., *Propionibacterium* sp. and *Malassezia* sp. were reported as the major bacteria and fungi associated with scalp diseases.[9] However, balance of *M. restricta* with other species is more important than existence of *M. restricta* in disease scalp. Xu et al.[7] reported that bacteria had a stronger relationship with the severity of dandruff than fungi. These results imply that it is more important to study the entire microbial community of the scalp than to study some specific bacteria and fungi.

Lower network density on diseased group in network analysis showed a correlation of the collapse of microbial community equilibrium with disease occurrence (Figure 2). This bacterial and fungal network disequilibrium (collapse) may be a main causation and/or consequences of D/SD. Our results will not only provide insight into finding the key-stone species associated with scalp diseases but also will suggest an importance of maintaining entire bacterial and fungal communities stability.

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CONFLICT OF INTERESTS

This manuscript has not been submitted for publication elsewhere, and the authors have no conflict of interests to declare. SA, TP, HGL, IK and JL were employees of Amorepacific Co.

Keywords

dandruff, next generation sequencing, scalp microbiome, seborrheic dermatitis

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REFERENCES

[1] A. K. Gupta, R. Batra, R. Bluhm, T. Boekhout, T. L. Dawson Jr, *J. Am. Acad. Dermatol.* **2004**, *51*, 785.
[2] L. Wang, C. Clavaud, A. Bar-Hen, M. Cui, J. Gao, Y. Liu, C. Liu, N. Shibagaki, A. Gueniche, R. Jourdain, K. Lan, C. Zhang, R. Altmeyer, L. Breton, *Exp. Dermatol.* **2015**, *24*, 398.
[3] E. A. Grice, H. H. Kong, S. Conlan, C. B. Deming, J. Davis, A. C. Young, G. G. Bouffard, R. W. Blakesley, P. R. Murray, E. D. Green, M. L. Turner, J. A. Segre; NISC Comparative Sequencing Program, *Science* **2009**, *324*, 1190.
[4] G. I. Perez Perez, Z. Gao, R. Jourdain, J. Ramirez, F. Gany, C. Clavaud, J. Demaude, L. Breton, M. J. Blaser, *PLoS One* **2016**, *11*, e0151990.
[5] L. Misery, N. Rahhali, A. Duhamel, C. Taieb, *Acta Derm. Venereol.* **2013**, *93*, 80.
[6] S. Ranganathan, T. Mukhopadhyay, *Indian J. Dermatol.* **2010**, *55*, 130.
[7] Z. Xu, Z. Wang, C. Yuan, X. Liu, F. Yang, T. Wang, J. Wang, K. Manabe, O. Qin, X. Wang, Y. Zhang, M. Zhang, *Sci. Rep.* **2016**, *6*, 24877.
[8] C. Clavaud, R. Jourdain, A. Bar-Hen, M. Tichit, C. Bouchier, F. Pouradier, C. E. I. Rawadi, J. Guillot, F. Menard-Szczebara, L. Breton, J.-P. Latge, I. Mouyna, *PLoS One* **2013**, *8*, e58203.
[9] H. K. Park, M. H. Ha, S. G. Park, M. N. Kim, B. J. Kim, W. Kim, *PLoS One* **2012**, *7*, e32847.

SUPPORTING INFORMATION

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FIGURE S1 Relative abundance of (A) bacterial phyla and (B) fungal species

FIGURE S2 MDS plots of (A) bacterial OTUs (OOB estimate of error rate: 28.43%, ntree=500), (B) fungal species (OOB estimate of error rate: 30.39%, ntree=500) and (C) bacterial OTUs and fungal species combined (OOB estimate of error rate: 27.45%, ntree=500)

FIGURE S3 Relative abundance of bacterial OTUs and fungal species having average relative abundance over 0.01% in Gini index top 100

FIGURE S4 Factors causing significant differences between normal and diseases groups. Especially, the disease groups had higher active folliculitis and pH but a lower hydration.

DATA S1 Materials and methods.

Data S2 Supplementary references

Table S1 Sample metadata, number of sequences and α-diversity across samples

Table S2 The distribution of survey data using ANOSIM

Table S3 The distribution of survey data using ANOVA

Table S4 Candidates bacteria causing disease symptoms

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In silico prediction of *Leishmania major*-specific CD8$^+$ epitopes

Abstract

Infections with *Leishmania (L.) major* induce protective IFN-γ-dependent Th1/Tc1 immunity in C57BL/6 mice as well as in immunocompetent humans. Even though antigen-specific immunity provides lifelong immunity against reinfection, a vaccine against this pathogen does not yet exist. Here, we compared the results obtained from in silico predictions of murine CD8-specific *L. major* peptides using the algorithm SYFPEITHI with the number and predicted affinity of known proteins/peptides. Our results indicate that the majority of "immunodominant" epitopes of *L. major* have not been identified so far; thus, computer-based prediction algorithms may aid the development of an effective vaccine.

1 | BACKGROUND

Leishmaniasis is a parasitic disease affecting 540 million people worldwide, with 1.3 million new cases reported annually and about 30 000 deaths per year.[S1] In immune competent hosts, for example humans or C57BL/6 mice, infection with *L. major* results in CD4$^+$ Th1/CD8$^+$ Tc1 immunity with long-lasting resistance, whereas in BALB/c mice or immune-suppressed humans, progressive, disseminated disease associated with Th2/Treg/Th17-driven immune response is observed.[1,2] Together with CD4$^+$ T cells, IFN-γ-producing CD8$^+$ Tc1 cells play an important role against *L. major* infections.[3] In endemic areas, "leishmanization" with viable parasites is performed to protect humans against re-challenge with the same *Leishmania* subspecies.[4,5] However, this procedure is associated with severe consequences and a vaccine against this important human pathogen is not yet available. In the past years, various single peptides, proteins and protein combinations have been tested in vitro and in vivo for their capacity to serve as a vaccine—most of them in mice. The most commonly analysed proteins are gp63, PSA, KMP-11, LACK, CPA, histone H1 and the polyprotein Leish-111f consisting of LeiF, TSA and LmST1. All these proteins were able to improve infection outcome in resistant C57BL/6 and/or susceptible BALB/c mice, but unfortunately were not able to induce long-lasting immunity. Strikingly, while the identification of *L. major*-specific proteins/peptides should be feasible based on the fact that the entire genome of *L. major* was sequenced,[S2] the relevant immunogenic peptides recognized by Th1/Tc1 cells are mostly unknown.

2 | QUESTION ADDRESSED

Within the present study, we now focused on predicting MHC I-restricted peptides that could be used as vaccine candidates in resistant C57BL/6 mice. This in turn would aid the development of a vaccine against human cutaneous leishmaniasis.

3 | EXPERIMENTAL DESIGN

The entire genome of *L. major* encodes for 8265 proteins (Figure S1).[S2] First, we evaluated all 8-mer and 9-mer peptides derived from these proteins for their predicted affinity to bind to C57BL/6-specific MHC I-molecules using the computer-based algorithm SYFPEITHI.[6] Resistant

Abbreviations: BG, Beatrix Grewe; CPA, cysteine proteinase a; DC, dendritic cell(s); EvS, Esther von Stebut; gp63, glycoprotein 63; GvZ, Ger van Zandbergen; HGR, Hans-Georg Rammensee; IFN-γ, interferon γ; JK, Joerg Kuharev; KDS, Kirsten Dietze-Schwonberg; KMP-11, kinetoplastid membrane protein-11; *L. major*, *Leishmania major*; LACK, *Leishmania* homologue of receptors for activated C kinase; LeiF, *Leishmania* homologue of eukaryotic ribosomal initiation factor 4a; LmST1, *L. major* homologue of the eukaryotic stress-inducible protein-1; LN, lymph nodes; MΦ, macrophages; PSA, promastigote surface antigen-2; SB, Sven Brosch; ST, Stefan Tenzer; Tc1, cytotoxic T cells; Th1, T-helper 1 cells; Treg, regulatory T cells; TSA, thiol-specific antioxidant protein.