

Original Article



Clinical Efficacy and Scalp Microbiome Changes Induced by AMPamide-Containing Shampoo in Patients With Seborrheic Dermatitis

Yi Na Yoon ¹, Sae Hee Kim ¹, Ji Won Lim ¹, Myeong Jae Kim ^{2,3},
Hye-Jin Kim ², Woo Jun Sul ², Daehwan Kim ⁴, Wonseok Jeong ⁴,
Jeonghwan Hwang ⁴, Da-Ae Yu ¹, Yong Beom Choe ¹, Yang Won Lee ¹

¹Department of Dermatology, Konkuk University School of Medicine, Seoul, Korea

²Department of Systemic Biotechnology, Chung-Ang University, Anseong, Korea

³Department of Dermatology, Incheon St. Mary's Hospital, College of Medicine, The Catholic University of Korea, Seoul, Korea

⁴CRID Center, Neopharm Co., Ltd., Daejeon, Korea



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Corresponding Author:

Yang Won Lee

Department of Dermatology, Konkuk
University School of Medicine, 120-1
Neungdong-ro, Gwangjin-gu, Seoul 05030,
Korea.

Email: 20050078@kuh.ac.kr

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ABSTRACT

Background: Seborrheic dermatitis (SD) is a chronic inflammatory scalp disorder associated with *Malassezia* dysbiosis and increased sebum production. AMPamide has been suggested to have anti-inflammatory and sebum-regulating effects, but its clinical efficacy and microbiome-modulating effects in SD remain unclear.

Objective: To evaluate the clinical efficacy and scalp microbiome changes following 4 weeks of use of an AMPamide-containing shampoo in patients with SD.

Methods: In this observational study, 30 patients with SD applied an AMPamide-containing shampoo for 4 consecutive weeks. Clinical outcomes, including sebum levels and overall severity scores, were assessed. Scalp bacterial and fungal communities were analyzed to evaluate α - and β -diversity and changes in *Malassezia* composition.

Results: Treatment resulted in significant reductions in sebum levels and clinical severity scores, particularly in erythema, dandruff, and pruritus. Bacterial community composition remained largely stable, while fungal α -diversity increased, and β -diversity analysis revealed a decrease in the ratio of *Malassezia restricta* to *Malassezia globosa*.

Conclusion: AMPamide-containing shampoo was associated with improved clinical symptoms and a shift toward a more balanced fungal community composition in patients with SD, supporting its potential as a non-steroidal therapeutic option for SD.

Keywords: Bacteria; Dandruff; Fungi; Microbiota; Seborrheic dermatitis

INTRODUCTION

Seborrheic dermatitis (SD) is a chronic inflammatory skin disorder affecting sebaceous gland-rich areas, characterized by erythematous patches with greasy scales that significantly affect patients' quality of life¹.

Recent microbiome studies have further suggested that SD involves not only species-level changes in *Malassezia* but also complex interactions between bacterial and fungal communities²⁻⁸. Reduced overall microbial diversity and disrupted network connectivity have been observed in the scalp of SD patients, indicating ecological instability of the scalp ecosystem^{2,9}. In particular,

the balance between *Malassezia restricta*—frequently dominant in lesional scalp—and *Malassezia globosa*—commonly found in healthy scalp—has been proposed as a key ecological marker distinguishing disease from health^{10,12}. Restoration of this fungal equilibrium, accompanied by increased community diversity, is thought to reflect a shift toward a healthy scalp state. However, longitudinal data describing how these microbial communities respond to treatment remain limited, leaving the ecological trajectory of SD recovery poorly understood.

The management of scalp SD typically involves topical treatments such as antifungals and anti-inflammatory agents. Antifungal treatments like ketoconazole are effective in inhibiting *Malassezia*, a key pathogen in SD. Various topical azole antifungals, such as ketoconazole, clotrimazole, and miconazole, have been proven effective in managing SD¹³. Topical corticosteroids (TCS), often used for their anti-inflammatory properties, can effectively address acute flares. However, long-term use of TCS is associated with several side effects, including telangiectasias, skin thinning, striae, and perioral dermatitis¹³. Therefore, there is a clinical need for non-steroidal therapeutic options that are safe for long-term use in patients with chronic and relapsing SD.

AMPamide is a novel non-steroidal compound with therapeutic potential for treating SD, as demonstrated in an *in vitro* study¹⁴. It targets both inflammation and sebogenesis by inhibiting the activation of Toll-like receptor (TLR) 4/6 receptors, which suppresses the production of pro-inflammatory cytokines, including interleukin (IL)-1 β , IL-6, and tumor necrosis factor- α , through the nuclear factor- κ B and mitogen-activated protein kinase pathways¹⁴. Furthermore, AMPamide reduces excessive lipid production in insulin-like growth factor-1-stimulated sebaceous cells by downregulating key lipid-regulating factors such as sterol regulatory element binding protein (SREBP)-1, peroxisome proliferator-activated receptor (PPAR)- γ , and stearyl-coenzyme A desaturase-1¹⁴. Unlike conventional antifungal or corticosteroid agents, AMPamide does not exert direct antimicrobial effects but may influence the scalp microbiome indirectly by modifying sebum composition and inflammatory signaling. This property makes it a promising, microbiome-conscious therapeutic approach for restoring ecological balance in SD.

In this observational study, we investigated the clinical efficacy and the effect of an AMPamide-containing shampoo on the scalp microbiota over a 4-week period in adult patients with scalp SD. This study aims to provide preliminary clinical evidence supporting the use of AMPamide as a potential therapeutic agent in the management of SD.

MATERIALS AND METHODS

Patients

This observational study included 30 adults with scalp SD who visited Konkuk University Medical Center between April and September 2024. Diagnosis was operationally defined as a clinical severity score (CSS) of ≥ 3 , calculated as the sum of 4 parameters—erythema, dandruff, pruritus, and the lesion extent (each rated 0–3; **Supplementary Table 1**). Patients who had received systemic or topical antibiotics, corticosteroids, immunosuppressants, antihistamines, retinoids, or phototherapy within 4 weeks prior to the screening visit were excluded. All participants provided written informed consent, and the study protocol was approved by the Institutional Review Board (IRB) of Konkuk University Medical Center (IRB No. 2023-12-004).

Test product

The formulation of the new shampoo containing AMPamide (INCI Name: Caproyl Methyl Serinate; Neopharm Co., Ltd., Daejeon, Korea) is presented in **Supplementary Table 2**. Participants were instructed to massage the assigned shampoo into the scalp for 1 to 2 minutes, followed by thorough rinsing with water, at least 5 times per week for 4 weeks. The use of other medications or topical agents for dermatologic conditions was not permitted during the study period.

Clinical assessments

Clinical visits were scheduled at Week 0, Week 2, and Week 4. At each visit, CSS was evaluated, and sebum levels were measured at 3 standardized scalp regions (frontal, vertex, and temporal) using a sebumeter (MPA5; Courage and Khazaka Electronic, Cologne, Germany) (**Fig. 1**).

Scalp microbiome and mycobiome analysis

1) Sample preparation

Scalp microbiome samples (n=89; one excluded due to low yield) were collected at the vertex using sterile rayon swabs. Only the swab heads were aseptically cut off and placed into 2 ml screw tubes (BioD, Gwangmyeong, Korea). The collected samples were stored at -80°C until genomic DNA (gDNA) extraction.

2) gDNA extraction

Bacterial and fungal gDNA extraction was performed using the Invitrogen PureLink™ Genomic DNA Mini Kit (Life Technologies, Carlsbad, CA, USA) in combination with 5 mm stainless steel beads. Each swab sample was mixed with 400 μl of lysis buffer containing lysozyme (20 mg/ml) and incubated at 37°C for 1 hour. Then, 45 μl of Proteinase K (20 mg/ml) and 445 μl of PureLink™ Genomic Lysis/Binding Buffer were added, followed

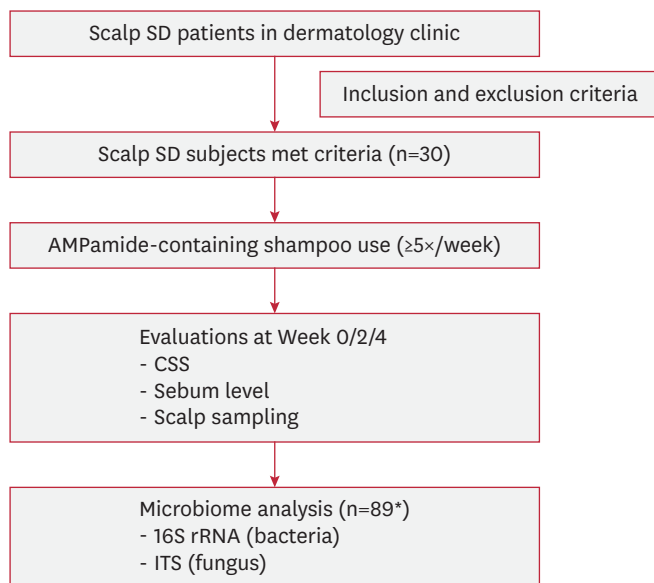


Fig. 1. Flow chart of the study process.

SD: seborrheic dermatitis, CSS: clinical severity score, ITS: internal transcribed spacer.

*One sample was excluded from the microbiome analysis due to low genomic DNA extraction yield.

by brief vortexing. Two stainless steel beads were added to each tube, and bead beating was performed for 1 minute using a Mini-Beadbeater-16 (BioSpec Products, Bartlesville, OK, USA). Samples were cooled on ice for 10 minutes and then kept at room temperature for an additional 10 minutes, followed by incubation at 55°C for 30 minutes. After washing with PureLink™ Genomic Wash Buffers 1 and 2, the gDNA was eluted with 31 µl of PureLink™ Genomic Elution Buffer. DNA concentration was measured using a Qubit™ 4 Fluorometer (Thermo Fisher Scientific, Waltham, MA, USA).

3) Target gene amplification and sequencing

Bacterial 16S rRNA gene amplification and sequencing

For bacterial community analysis, the full-length 16S rRNA gene (V1–V9 regions) was amplified. The primer sequences used were as follows: 16S rRNA degenerate forward primer sequence (5'-GCATC/barcode/AGRGTTYGATYMTGGCTCAG-3') and 16S rRNA degenerate reverse primer sequence (5'-GCATC/barcode/RGYTACCTTGTTACGACTT-3'). Amplification was performed using KAPA HiFi HotStart ReadyMix (Kapa Biosystems, Wilmington, MA, USA) with 5 µl of template gDNA (1–2 ng). The reaction conditions included an initial denaturation at 95°C for 3 minutes, followed by 30 cycles of amplification (denaturation at 95°C for 30 seconds, annealing at 57°C for 30 seconds, and extension at 72°C for 1 minute) and a final extension step at 72°C for 5 minutes. Sequencing was performed using the PacBio Revio

platform employing Single Molecule Real-Time sequencing technology to repeatedly read individual DNA molecules, generating highly accurate circular consensus sequence (CCS) reads. These CCS reads were used for subsequent analyses.

Fungal internal transcribed spacer 1 (ITS1) region amplification and sequencing

For fungal community analysis, the ITS1 region was targeted for amplification. The primers used were as follows: ITS1 18S-F (GTAAAAGTCGTAACAAGGTTTC) and ITS1 5.8S-1R (GTTCAAAGAYTCGATGATTAC). The amplification reaction was performed using KAPA HiFi HotStart ReadyMix, 5 µl each of the forward and reverse primers (1 µM), and 2.5 µl of template gDNA (5 ng/µl). The reaction conditions included an initial denaturation at 95°C for 3 minutes, followed by 33 cycles of amplification (denaturation at 95°C for 30 seconds, annealing at 55°C for 30 seconds, and extension at 72°C for 1 minute) and a final extension step at 72°C for 5 minutes. After purification with AMPure XP beads (Beckman Coulter, High Wycombe, UK), an index polymerase chain reaction (PCR) was conducted using the Nextera XT Index Kit (N716–N729/S502–S511). The PCR conditions were the same as those described above, except that the repeat step was set to 8 cycles. The index PCR products were purified again, quantified with a Qubit™ 4 Fluorometer, and pooled based on the quantification results. Finally, paired-end sequencing (2×300 bp) was performed using the Illumina MiSeq™ platform (Illumina, San Diego, CA, USA).

4) Sequence data processing and analysis using the Quantitative Insights Into Microbial Ecology 2 (QIIME 2) pipeline

Raw sequencing data obtained from the PacBio Revio and Illumina MiSeq platforms were processed and analyzed using the QIIME 2 pipeline¹⁵ and the R program (R Foundation for Statistical Computing, Vienna, Austria).

Processing of bacterial sequence data

Raw bacterial reads were preprocessed using the DADA2¹⁶ plugin (dada2 denoise-ccs) to generate amplicon sequence variants (ASVs). Taxonomic classification was conducted using the feature-classifier classify-sklearn¹⁷ plugin, which employs a Naive Bayes classifier trained on the Greengenes2 database¹⁸. ASVs classified as non-bacterial sequences (e.g., chloroplast or mitochondria) were excluded from downstream analyses. The remaining ASVs were aligned using MAFFT¹⁹ and a phylogenetic tree was constructed using the align-to-tree-mafft-fasttree²⁰ pipeline. A total of 9,298,948 raw reads were obtained (average 104,483 reads per sample) and 3,525 ASVs were identified after filtering. For normalization, sequences were rarefied to 5,540 reads per sample.

Processing of fungal sequence data

Fungal reads were trimmed with Cutadapt²¹ plugin and processed with the DADA2 plugin (dada2 denoise-paired) to produce ASVs. Taxonomic classification was performed using the UNITE database²², and unclassified ASVs were excluded from further analyses. The remaining ASVs were aligned using MAFFT, and a phylogenetic tree was generated as described above. A total of 19,485,622 raw reads were generated (average 218,940 reads per sample), yielding 3,539 ASVs. For normalization, sequences were rarefied to 29,141 reads per sample.

Statistical analysis

For the CSS, changes from baseline were analyzed and data were summarized as the mean $\pm$ standard deviation, along with the minimum, maximum, and the 95% confidence intervals of the changes. To account for within-subject correlation, paired t-tests or Wilcoxon signed-rank tests were used to compare the CSS scores and sebum levels. Statistical analyses were performed using SPSS (IBM Corp., Armonk, NY, USA) and SAS software (SAS Institute, Cary, NC, USA), with a significance level of $p \leq 0.05$ considered statistically significant for all tests.

To evaluate changes in the scalp microbial diversity, both alpha and beta diversity analyses were performed. Alpha diversity was assessed using Faith's phylogenetic diversity, which accounts for the evolutionary distances, and the Shannon index, reflecting both species richness and evenness. Statistical analysis for comparing alpha diversity was performed using the Kruskal-Wallis test or one-way analysis of variance, followed by Dunn's *post hoc* test adjusted by the Benjamini-Hochberg correction. Beta diversity was calculated based on UniFrac distances; unweighted UniFrac evaluated community composition based on presence/absence (sensitive to rare species), while weighted UniFrac incorporated relative abundance to capture shifts in dominant populations. Microbial community differences across visits were visualized by principal coordinates analysis (PCoA). To evaluate significant differences in microbial community composition among groups, analysis of similarities (ANOSIM) was conducted using the diversity beta-group-significance plugin in QIIME 2. Microbial taxonomic composition was examined using the QIIME 2 taxa bar-plot plugin, and visualizations were generated using R.

RESULTS

Clinical outcomes

1) Demographic and clinical characteristics

A total of 30 patients were enrolled in the study, of whom 20 (67%) were female. The mean age of participants was 32 ± 9 years. At baseline (Week 0), the mean CSS was 7.2 ± 1.4 , and the mean

sebum level was 127.1 ± 60.5 . Sebum measurements obtained from different scalp regions—frontal, vertex, and temporal—showed the highest value at the frontal region (frontal: 140.2 ± 68.2 ; vertex: 126.6 ± 75.5 ; temporal: 114.4 ± 59.9). Among all participants, one patient (3%) had a concomitant diagnosis of atopic dermatitis.

2) CSS

Changes in the CSS among patients are presented in **Table 1**. The mean CSS significantly decreased through Week 4 (3.60 ± 2.06 , $p < 0.001$). Significant improvements were noted in all individual components of the CSS.

3) Sebum level

Changes in sebum levels among patients are presented in **Table 2**. The mean sebum level significantly decreased after 2 weeks of treatment (Week 2; $88.52 \pm 49.99 \mu\text{g}/\text{cm}^2$, $p < 0.001$) and continued to decrease at Week 4 ($66.52 \pm 43.25 \mu\text{g}/\text{cm}^2$, $p < 0.001$). Specifically, the sebum levels in all regions showed a significant reduction at both Week 2 and Week 4.

Scalp microbiome

1) Diversity of bacterial and fungal communities

We evaluated both bacterial and fungal community diversity across the 3 time points (Week 0, Week 2, and Week 4) using α - and β -diversity analyses. For α -diversity, Faith's phylogenetic diversity and Shannon index were calculated (**Fig. 2**). In the bacterial community, Faith's phylogenetic diversity revealed a statistically significant increase from Week 2 to Week 4 ($p < 0.05$; **Fig. 2A**), whereas no significant differences were observed among visits based on the Shannon index (**Fig. 2B**). In contrast, fungal diversity assessed by the Shannon index showed significant differences

Table 1. Changes in the clinical severity score over 4 weeks of application of AMPamide-containing shampoo in patients with seborrheic dermatitis

Clinical parameter	Week 0	Week 2	Week 4
Erythema (0–3)	1.73 ± 0.58	$1.30 \pm 0.53^*$	$0.87 \pm 0.73^*$
Dandruff (0–3)	1.60 ± 0.62	$1.17 \pm 0.53^{**}$	$0.83 \pm 0.70^*$
Pruritus (0–3)	1.63 ± 0.67	$1.23 \pm 0.50^{***}$	$0.60 \pm 0.62^*$
Lesion extent (0–3)	2.20 ± 0.48	$1.73 \pm 0.69^*$	$1.30 \pm 0.79^*$
Total (0–12)	7.17 ± 1.42	$5.43 \pm 1.55^*$	$3.60 \pm 2.06^*$

Values are presented as mean $\pm$ standard deviation. Comparison to Week 0; * $p < 0.001$, ** $p < 0.002$, *** $p < 0.003$.

Table 2. Changes in sebum levels at the frontal, vertex, and temporal scalp regions over 4 weeks of application of AMPamide-containing shampoo in patients with seborrheic dermatitis

Region	Week 0	Week 2	Week 4
Frontal	140.20 ± 68.16	$98.77 \pm 59.57^*$	$80.80 \pm 52.38^*$
Vertex	126.60 ± 75.53	$85.17 \pm 54.96^*$	$60.63 \pm 45.52^*$
Temporal	114.37 ± 59.86	$81.63 \pm 53.31^*$	$58.13 \pm 47.66^*$
Mean	127.06 ± 60.45	$88.52 \pm 49.99^*$	$66.52 \pm 43.25^*$

Values are presented as mean $\pm$ standard deviation. Comparison to Week 0; * $p < 0.001$.

between Week 0 and Week 2, as well as between Week 0 and Week 4 ($p < 0.001$ and $p < 0.01$, respectively; **Fig. 2D**), while Faith's phylogenetic diversity did not differ significantly among visits (**Fig. 2C**).

β -diversity analysis further revealed temporal changes in microbial community composition associated with the use of

the AMPamide-containing shampoo. PCoA based on UniFrac distances showed distinct clustering patterns over the 3 visits (**Fig. 3**). For the bacterial community, PCoA based on unweighted UniFrac distance demonstrated a significant difference between Week 2 and Week 4 (ANOSIM, $p < 0.005$; **Fig. 3A**), whereas no significant

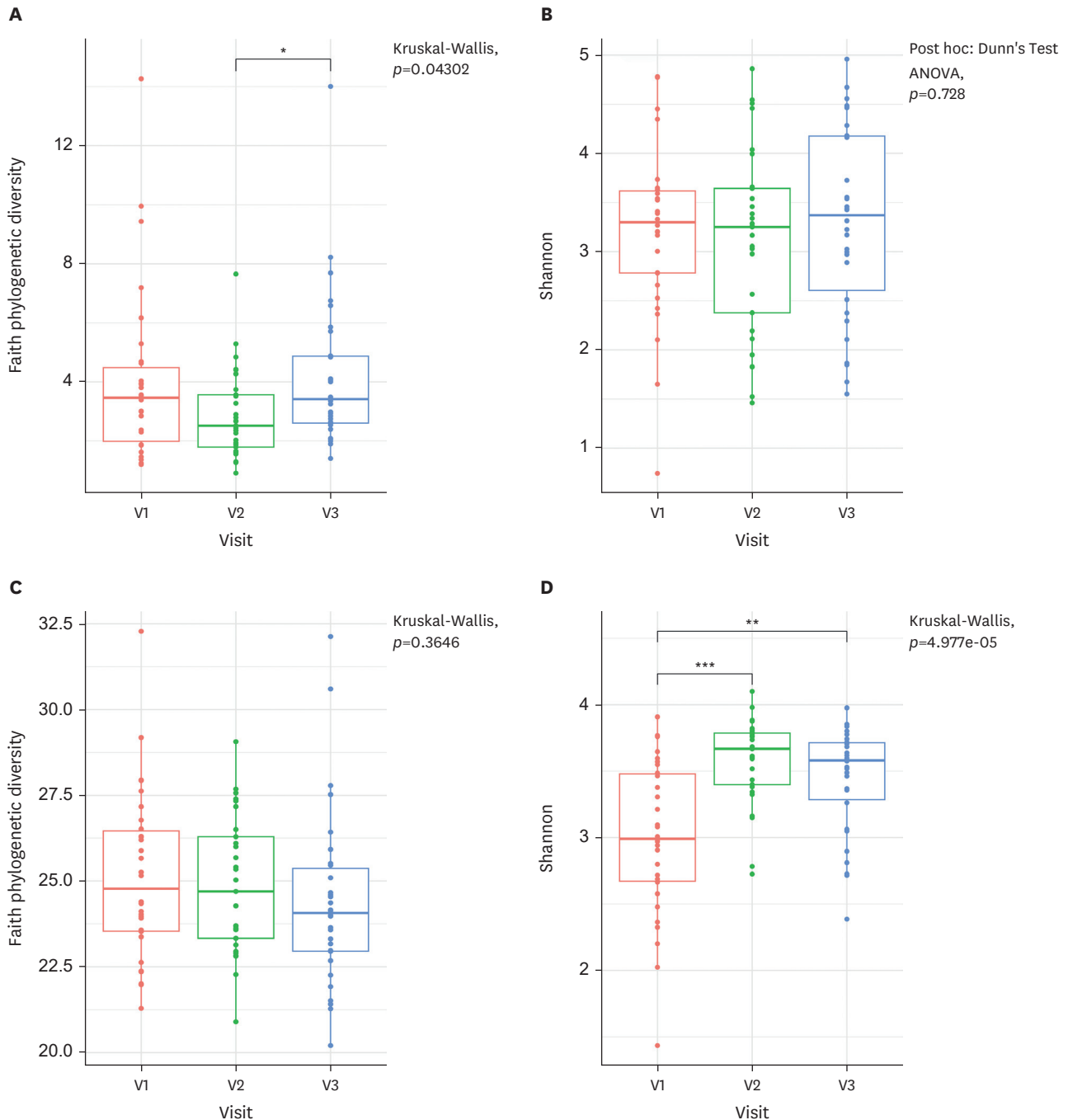


Fig. 2. Alpha diversity of bacterial (A, B) and fungal (C, D) communities. Faith's phylogenetic diversity (A, C) and Shannon index (B, D) were used to assess alpha diversity. Statistical analysis was performed using the Kruskal-Wallis test or one-way ANOVA with Dunn's *post hoc* test adjusted by Benjamini-Hochberg correction. ANOVA: analysis of variance.

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, not significant: > 0.05 .

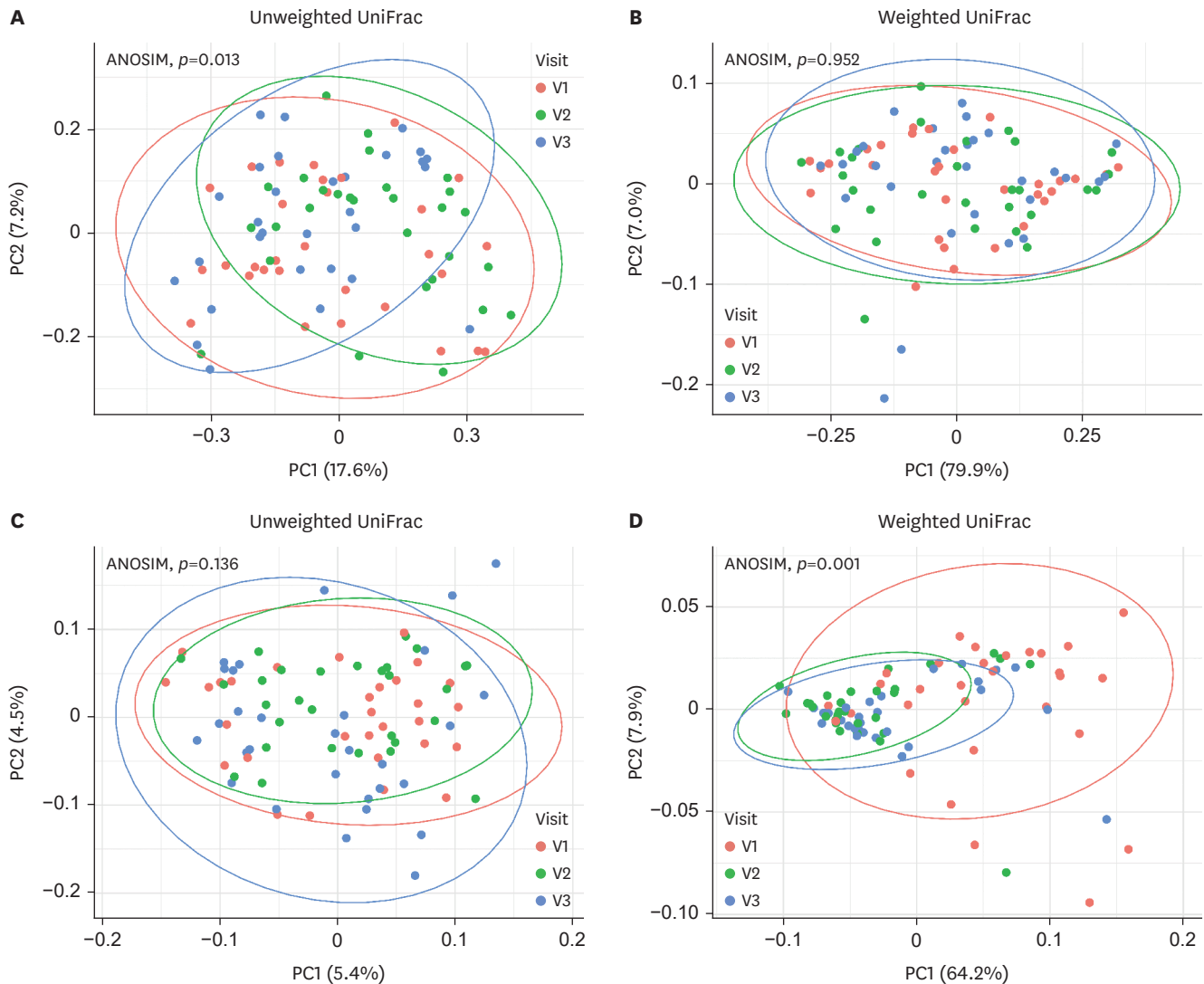


Fig. 3. Principal coordinates analysis plots of bacterial (A, B) and fungal (C, D) communities based on unweighted (A, C) and weighted UniFrac distances (B, D). To evaluate significant differences in microbial community composition among groups, ANOSIM was conducted. ANOSIM: analysis of similarities, PC: principal component.

separation was observed when weighted UniFrac distance was applied (**Fig. 3B**). Regarding the fungal community, PCoA based on unweighted UniFrac distance did not reveal significant differences among visits (**Fig. 3C**). However, analysis using weighted UniFrac distance showed clear separations between Week 0 and Week 2, and between Week 0 and Week 4 ($p<0.001$ for both; **Fig. 3D**).

2) Changes in bacterial and fungal taxonomic composition

The bacterial community composition of the scalp was characterized at multiple taxonomic levels (phylum to species) across the 3 visits (**Fig. 4, Supplementary Fig. 1**). At the phylum level, Actinomycetota (mean 54.67%) and Bacillota_I (43.83%) were consistently dominant among participants across all

visits (**Supplementary Fig. 1A**). Overall, the bacterial community remained largely stable throughout the study period, with no significant changes observed at the class, order, family, or genus levels (**Supplementary Fig. 1**). Minor phyla such as Pseudomonadota and Bacteroidota collectively represented less than 2% of the total bacterial community. At the species level, no statistically significant temporal variation was observed (permutational analysis of variance, $p=0.851$, $R^2=0.0036$), and inter-individual variability exceeded intra-individual temporal differences (**Fig. 4A and B**).

For the fungal community, compositional profiles from phylum to species levels showed a clear predominance of Basidiomycota (91.42%) over Ascomycota (6.25%; **Supplementary Fig. 2A**). Across lower taxonomic ranks, Malasseziomycetes, Malasseziales, Malasseziaceae, and *Malassezia* were consistently dominant

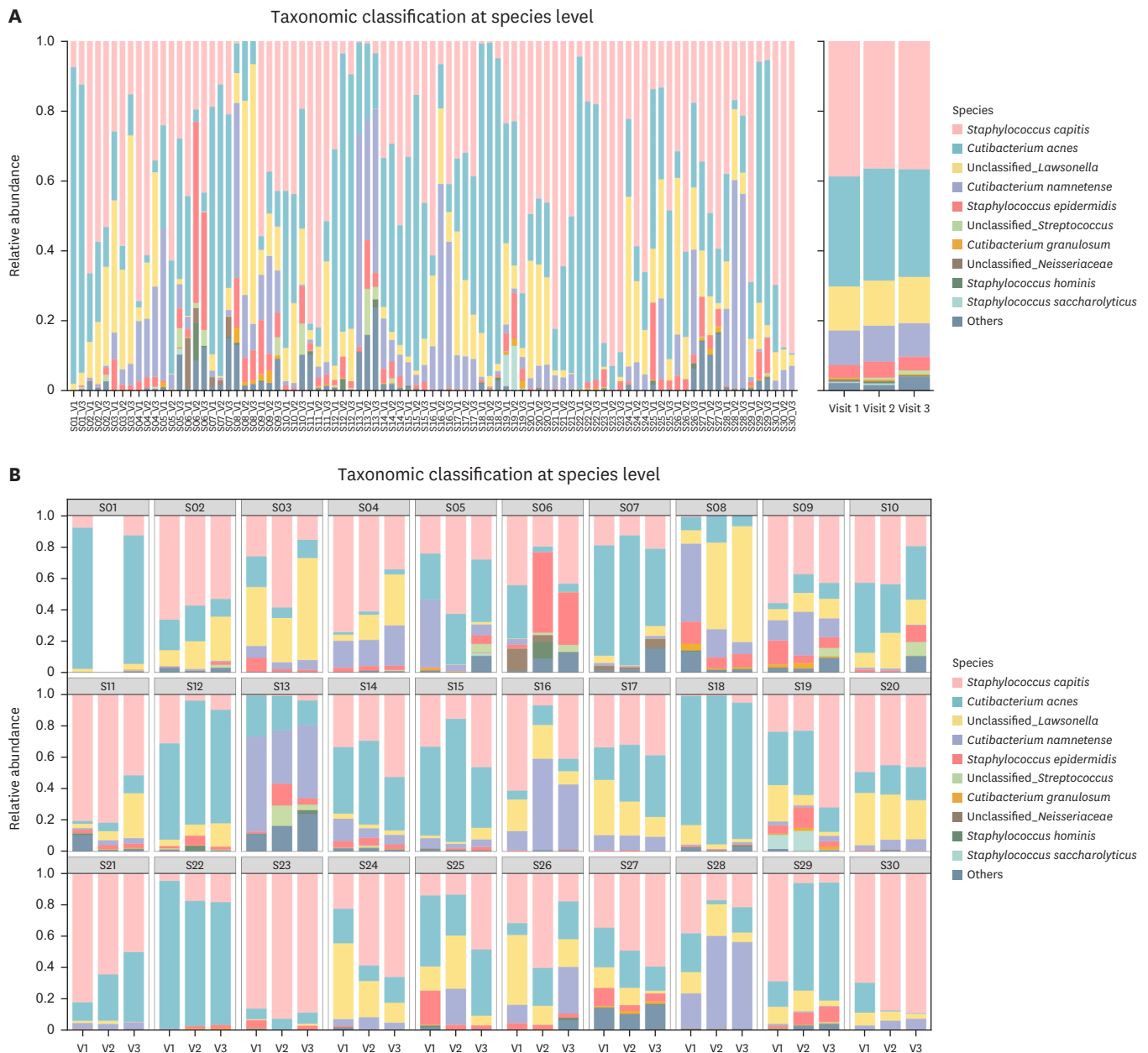


Fig. 4. Taxonomic compositions of the bacterial (A, B) and fungal (C, D) communities at the species level. The relative abundances of the top 10 species are shown. (continued to the next page)

(**Supplementary Fig. 2B-E**). At the species level, *M. restricta* (43.41%), *M. globosa* (26.03%), and unclassified *Malassezia* spp. were predominant in all samples (**Fig. 4C and D**). When averaged by visit, the relative abundance of *M. restricta* showed a decreasing trend, while *M. globosa* increased at Week 2 and Week 4 compared to Week 0 (Wilcoxon signed-rank test, $p=3.63 \times 10^{-5}$ and 1.56×10^{-5} , respectively; **Fig. 4C**).

Individual-level analyses revealed substantial inter-individual variability in fungal composition across visits (**Fig. 4D**). Nevertheless, the majority of participants exhibited a consistent

pattern of decreasing *M. restricta* and increasing *M. globosa* abundance from Week 0 to Week 4, suggesting a potential community-level shift within *Malassezia* species in response to repeated AMPamide-containing shampoo exposure.

DISCUSSION

In this observational study, we evaluated the clinical efficacy and changes in the scalp microbiome in patients with SD after using



Fig. 4. (Continued) Taxonomic compositions of the bacterial (A, B) and fungal (C, D) communities at the species level. The relative abundances of the top 10 species are shown.

an AMPamide-containing shampoo for 4 consecutive weeks. We found that the use of AMPamide-containing shampoo significantly improved the symptoms of SD by reducing sebum levels and overall CSSs. Additionally, the ratio of *M. restricta* to *M. globosa* in the fungal community decreased, while the bacterial community composition did not show any significant change in the microbiome analysis.

The clinical improvements, particularly in redness, scaling, and itching, are consistent with the anti-inflammatory effects

and anti-sebogenic properties of AMPamide. AMPamide suppresses TLR4/6-mediated inflammatory signaling pathways and downregulates the expression of factors related to lipid formation and sebum secretion, such as SREBP-1 and PPAR- γ ¹⁴. *Malassezia* lipases hydrolyze sebum-derived lipids such as triglycerides and squalene into pro-inflammatory fatty acids, which contribute to the erythema, scaling, and pruritus in SD²³⁻²⁵. By modulating inflammatory signaling and sebum synthesis, AMPamide potentially reduces the reservoir of lipid substrates that *Malassezia*

metabolizes into pro-inflammatory fatty acids. This reduction plausibly explains the observed shift from *M. restricta* dominance toward a more balanced fungal composition. Given that dysregulated sebum secretion may be an important contributor to the development of SD, the inhibitory effects of AMPamide on lipid synthesis are proposed as a key mechanism underlying the significant reduction in sebum levels and the improvement of the clinical symptoms.

Results from a scalp microbiome study suggest that the use of AMPamide-containing shampoo is associated with selective ecological modulation of the scalp microbial community rather than a broad-scale compositional shift. The α -diversity of the bacterial community showed minimal temporal variation during product use, and the β -diversity analysis revealed that differences were mainly observed in low-abundance taxa, suggesting that the bacterial community was largely resilient throughout the treatment period. Such stability implies that bacterial dysbiosis is unlikely to be the primary driver of SD.

In contrast, the fungal community exhibited more pronounced and structured changes. Fungal α -diversity increased over the course of AMPamide-containing shampoo use, and β -diversity based on weighted UniFrac distances revealed distinct separations between baseline and post-treatment visits. These results indicate that the scalp mycobiome underwent an abundance-weighted restructuring, particularly within *Malassezia* species. The relative decrease in *M. restricta* and the concomitant increase in *M. globosa* are of particular interest. The observed shifts in microbial composition are particularly noteworthy in light of previous clinical data. A study conducted in Korea identified *M. restricta* as the most common *Malassezia* species in patients with SD, whereas *M. globosa* was more prevalent among healthy controls¹¹. *M. restricta* has been associated with inflammatory and lipid-dysregulating activity in SD lesions, whereas *M. globosa* predominates on healthy scalp^{6,12}. However, it is essential to acknowledge that the role of *Malassezia* species in SD is highly complex and cannot be explained solely by changes in individual species. While the observed taxonomic shift suggests a transition toward a healthier state, it may not necessarily constitute a definitive resolution of the underlying pathophysiology, as the actual disease process involves sophisticated interplay between the host's sebum composition and microbial metabolism. Nevertheless, the directional change observed in our study suggests that the scalp ecosystem became more stable following the use of AMPamide-containing shampoo, likely due to increased microbial diversity. Higher α -diversity is typically linked to greater ecological resilience, as diverse microbial networks can suppress the expansion of opportunistic species and maintain homeostasis through competitive and cooperative interactions²⁶. A similar pattern of diversity recovery was reported in a study showing that the scalp microbiome in patients

with SD and dandruff had a more disrupted microbial network than that of a healthy scalp⁶. Ultimately, these alterations likely reflect niche-level adaptation and ecological response to clinical symptom relief and a modified scalp environment.

Taken together, these findings suggest that the use of AMPamide-containing shampoo is associated with a trend toward re-establishing ecological balance within the scalp microbiome—particularly by increasing fungal diversity and reducing the dominance of *M. restricta*—without significantly perturbing the commensal bacterial population. By simultaneously reducing inflammatory signaling and sebum levels, the test product may indirectly contribute to reshaping the fungal ecosystem into a state more characteristic of a healthy scalp. The concordance between sebum reduction, clinical improvement, and the restructuring of the fungal community suggests a significant association between treatment and ecological shifts, though these findings remain preliminary regarding direct causality.

This was an observational study without a control group, and several limitations should be considered. First, the small sample size may lead to selection bias and prevent generalization to a larger population. Second, as this was an open-label observational study, the absence of a placebo or control group makes it difficult to definitively distinguish the treatment effects of the AMPamide-containing shampoo from the natural fluctuation of the disease course. Despite this limitation, our findings provide meaningful real-world evidence of clinical symptom improvement in SD during active treatment, which warrants further validation through randomized, double-blind, placebo-controlled trials. Third, as this study did not include metatranscriptomic or metabolomic analyses, the biochemical interplay between sebum reduction and *Malassezia* dynamics remains yet to be substantiated at a functional level. Our proposed mechanisms should be regarded as a conceptual framework that provides a basis for future mechanistic inquiries rather than definitive proof of metabolic pathways. Additionally, while AMPamide is the principal component targeting sebogenesis and inflammation, synergistic effects of other functional constituents including antioxidants and soothing agents cannot be definitively excluded. Future studies utilizing a vehicle-controlled design are necessary to isolate the specific therapeutic contribution of AMPamide. Furthermore, while paired tests were used, future studies could benefit from mixed-effects models to better analyze longitudinal variance. Finally, the short duration of the study limits long-term analysis of the sustainability of clinical and microbiome results.

In conclusion, this study provides preliminary clinical and microbiological evidence that AMPamide-containing shampoo is associated with a reduction in disease severity and shifts in the fungal microbiome toward a more balanced state in patients with SD. Given the chronic and relapsing nature of

SD and the safety concerns associated with long-term corticosteroid use, AMPamide is a potential candidate warranting further investigation for repeated or maintenance treatment. The observed decrease in the ratio of *M. restricta* to *M. globosa* suggests a therapeutic shift in the microbial composition and may represent a valuable biomarker for treatment response. These findings support the potential role of AMPamide as a novel non-steroidal agent for SD management and warrant further investigation in larger, long-term, controlled trials.

ORCID iDs

Yi Na Yoon 

<https://orcid.org/0000-0001-8050-1750>

Sae Hee Kim 

<https://orcid.org/0009-0003-1486-626X>

Ji Won Lim 

<https://orcid.org/0009-0000-2069-8053>

Myeong Jae Kim 

<https://orcid.org/0009-0002-2158-8483>

Hye-Jin Kim 

<https://orcid.org/0000-0001-6008-470X>

Woo Jun Sul 

<https://orcid.org/0000-0002-7016-1454>

Daehwan Kim 

<https://orcid.org/0009-0000-5677-0467>

Wonseok Jeong 

<https://orcid.org/0009-0008-9653-8900>

Jeonghwan Hwang 

<https://orcid.org/0009-0007-1401-8809>

Da-Ae Yu 

<https://orcid.org/0000-0001-8158-9766>

Yong Beom Choe 

<https://orcid.org/0000-0002-3074-7602>

Yang Won Lee 

<https://orcid.org/0000-0003-1268-1248>

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CONFLICTS OF INTEREST

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DATA SHARING STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

SUPPLEMENTARY MATERIALS

Supplementary Table 1

Criteria for clinical severity score

Supplementary Table 2

The ingredients of the new-formula shampoo with AMPamide

Supplementary Fig. 1

Taxonomic compositions of the bacterial communities at the phylum (A), class (B), order (C), family (D), and genus (E) levels. The relative abundances of the top 10 taxa are shown.

Supplementary Fig. 2

Taxonomic compositions of the fungal communities at the phylum (A), class (B), order (C), family (D), and genus (E) levels. The relative abundances of the top 10 taxa are shown.

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