

Effect of an emollient on the mycobiome of atopic dermatitis patients

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Introduction:

Atopic dermatitis (AD), is associated with changes in skin bacterial microbiome. Emollient treatment induces change in bacterial microbiome in AD, but its effect on fungal microbiome (“mycobiome”), and their inter-kingdom correlations is unknown. We used Ion-Torrent sequencing to characterize the mycobiome of AD patients in response to ET.

Methods:

Skin swabs were collected from lesional and non-lesional skin of AD patients suffering from moderate AD, after informed consent and according to GCP guidelines. Genomic DNA was extracted from each swab using the MoBio PowerSoil DNA Isolation, and used for mycobiome sequencing analyses as described in our earlier publications. Principal coordinates analyses (PCoA), diversity, abundance and correlations analyses were conducted in *R* and relevant packages, using non-parametric tests ($P < 0.05$ was significant).

Results:

Swab samples from 10 patients (7 females, 3 males; mean age, 10.5 years) were analyzed. Emollient treatment induced a significant reduction of Scoring Atopic Dermatitis (SCORAD) score ($P < 0.001$). PCoA showed pre-treatment and post-treatment samples clustered differently at all taxa levels. Six genera were detected in only non-lesional samples, while four were detected in only lesional samples. In non-lesional samples, Shannon diversity index was significantly increased after emollient

treatment ($P \leq .04$), while lesional skin exhibited non-significant decrease. Ascomycota was the most abundant phylum and Dothideomycetes was the most abundant Class in most samples. Eight fungal species were either significantly different ($P < .05$) or showed a strong trend ($P < .1$) between pre- and post-treatment samples of lesional and non-lesional skin. In lesional skin, Gram-negative *Pseudomonas* spp. correlated significantly with pathogenic fungal species (*Aspergillus*, *Candida* spp.) in pre-treatment samples; these correlations were not detected in post-treatment samples. Moreover, lesional skin exhibited significant correlations between Gram-positive bacteria (*Corynebacterium kroppenstedtii* and *Staphylococcus pettenkoferi*) and pathogenic *Candida* species in pre-treatment samples, but not in post-treated samples.

Discussion:

Emollient treatment may induce beneficial microbial changes in the mycobiome, and augment host-microbe balance on skin in AD. Clinical relevance of these results need to be investigated.

INTRODUCTION

Atopic dermatitis (AD) is a chronic inflammatory skin disorder that results in areas of dry, itchy skin.^{1,2} Several cultivation-dependent and –independent studies have identified changes in the composition of microbial communities in these affected areas over time and when compared to healthy control individuals.³⁻⁸ However, how these communities vary on affected and unaffected skin of the same individual, and how these communities respond to treatment, remains poorly understood.

In a previous study, Flores et al.⁹ characterized the bacterial microbiota of affected and unaffected skin of 49 patients with AD before and after emollient treatment using high-throughput sequencing V1-V2 areas of the 16S rRNA gene. These investigators showed that microbial diversity and community composition was different between affected and unaffected skin of AD patients prior to treatment. Differences were driven primarily by the overabundance of *Staphylococcus* species on affected skin and a corresponding decrease in bacterial diversity. After 3-months of emollient treatment, the clinical symptoms of AD improved in 72% of the study population and an average decreased SCORAD of -38% (from 34 to 21) was noted. Microbial communities associated with affected skin of these treatment responders more closely resembled unaffected skin after treatment as indicated by increased overall diversity and a decrease in the abundance of *Staphylococcus* species without any antibiotic treatment. Interestingly, *Xanthomonas* genus was significantly more abundant and *Staphylococcus* genus significantly less abundant in the communities of ‘responders’, suggesting a possible role of *Xanthomonas* in restoration of the skin microbiome in patients with AD.

Interestingly, those bacteria have been described to have enzymatic tools to digest keratin.

In the current “follow-up” study, we expanded our analyses of the microbiota by exploring the profile of the fungal microbiome (mycobiota) in these collected samples, using an Ion-Torrent-platform based analysis and amplification of the pan-fungal internally transcribed spacer (ITS) regions of the 18S rRNA gene.

METHODS

Study Design: DNA extracted from skin swabs collected from AD patients was obtained as described in our earlier publication.⁹ Briefly, this study protocol complied with the ethical guidelines of the 1975 Declaration of Helsinki, was approved by the DOST ethical committee, and conducted according to ICH guidelines for Good Clinical Practice. Patients were instructed to apply the emollient twice daily, once in the morning and once in the evening to their entire body. Patients were also instructed not to change their hygiene practices or to apply any other emollient (or any drugs including corticotherapy or antibiotherapy) during the study. This single center study included 49 patients (17 males and 32 females; aged 3 to 39 years) suffering from moderate AD. Clinical assessment of disease severity and microbiome sampling was conducted on two separate visits: day 0 and Day 84. the same investigating dermatologist evaluated severity with the SCORAD of one typical affected skin areas and a proximal unaffected site. Skin microbiome samples were collected using aseptic techniques under sterile air flow generated by a portable hood. Single use sterile cotton-tipped swabs (COPAN Ref.

165KS01) were pre-moistened with a sterile solution of deionized water containing 0.15 M NaCl and 0.1% Tween 20. Swabs were rubbed firmly for 20 seconds over 1cm² areas. Similarly, samples were also collected from the closest unaffected skin area. The cotton tip samples were stored at -80°. Genomic DNA (gDNA) was extracted from each swab using the MoBio PowerSoil DNA Isolation. Stored at -80°C during, this gDNA was used to describe here the global mycobiota picture. That means that the same DNA samples were used to describe bacteria and fungi global picture.

Microbiome Analysis

Analysis of the microbiome profile in the extracted DNA samples was conducted as described previously by our group,¹⁰⁻¹² and summarized below.

Amplicon Library Preparation: The Internal Transcribed Spacer 1 (ITS1) and 16S rDNA regions for fungi and bacteria, respectively, were amplified as described previously.^{10,12} Briefly, ITS1 region was amplified using ITS1F (CTTGGTCATTTAGAGGAAGTAA) and ITS 2 (GCTGCGTTCTTCATCGATGC) primers. The reactions were carried out on 100 ng template DNA, in 50 µl (final volume) reaction mixture consisting of Dream Taq Green PCR Master Mix (Thermo Scientific), 0.1g/L bovine serum albumin, 1% of dimethyl sulfoxide (DMSO), 6 mM MgCl₂, and a final primer concentration of 400nM. Initial denaturation at 94°C for 3 min was followed by 35 cycles of denaturation for 30s each at 94°C, annealing at 50°C for 30 s, and extension at 72°C for 1 min. Following the 35 cycles there was a final extension time of 5 min at 72°C. The PCR products were sheared for 20 min, using Ion Shear Plus

Fragment Library Kit (Life Technologies, NY, USA). The Amplicon library was generated with sheared PCR products using Ion plus Fragment Library kits (<350 bp) according to the manufacturer's instructions. The library was barcoded with Ion Xpress™ Barcode Adapter, and ligated with the A and P1 adaptors.

Sequencing, classification and analysis: The adapted barcoded libraries were equalized using the Ion Library Equalizer kit to a final concentration of 100 pM. Once equalized, the samples were pooled and diluted to 26 pM, and attached to the surface of Ion Sphere particles (ISPs) using an Ion PGM Template OT2 200bp kit v2 (Life Technologies, USA) according to the manufacturer's instructions, via emulsion PCR. Quality of ISPs templates was checked using Ion Sphere™ Quality Control Kit (Part no. 4468656) with the Qubit 2.0 device. Sequencing of the pooled libraries was carried out on the Ion Torrent Personal Genome Machine (PGM) system using the Ion Sequencing 200 kit v2 (all from Life Technologies) for 150 cycles (600 flows), with a 318 chip following the manufacturer's instructions. De-multiplexing and classification was performed using the Qiime 1.6 platform. Obtained sequencing data was cleaned to remove the barcodes, low quality reads (<75 bp, quality score<18), and primers during the de-multiplexing process. Bioinformatics process filters were applied to the sequence data for the removal of low-quality reads below Q25 Phred score and denoised to exclude sequences with read length below 100 bp.¹³ De novo operational taxonomic units (OTUs) were clustered using Uclust algorithm and defined by 97% sequence similarity.¹⁴ Classification at the species level was referenced using the UNITE 5.8s database and taxa assigned using the nBlast method with a 90% confidence cut-off.^{15,16}

Bioinformatics and Statistical Analyses: The statistical programming language *R* and related packages (*vegan*, *PMCMR*, *corrplot*) were used for principal coordinates analysis (PCoA), alpha/beta diversity, correlation analyses, and non-parametric analyses (since equal variance could not be assumed due to small sample size).¹⁷⁻²⁰ Taxa with frequency of at least 20% (and relative abundance >1%) were considered to comprise the core biome. PCoA was conducted with identified reads/OTUs using classical multidimensional scaling (functions *dist/euclidean*, *cmdscale*) to analyze distribution of dissimilarities and hierarchical clustering in dendrograms in the *R* package *vegan*. Homogeneity of groups and beta diversity was evaluated using *adonis* (which explores the differences in group means and implements a multivariate analysis of variances using distance matrices) and *betadisper* (which studies the differences in group homogeneities, similar to Levene's test of the equality of variances) functions in *vegan*. Diversity was analyzed using Shannon diversity index (characterizes species diversity) and abundance-based richness estimates (observed, bias-corrected Chao and ACE) were calculated using *vegan*.¹⁸ Taxa with a frequency of at least 20% (and relative abundance >1%) were considered to comprise the core biome in our study. Intra- and inter-kingdom correlations were determined using the *corrplot* package in *R*. Proportions of specific bacteria and fungi in tested samples were compared using non-parametric tests (*PMCMR* package. Due to small sample size, equal variance across data could not be assumed and data were analyzed using non-parametric tests followed by Dunn's test (Bonferroni post-hoc correction), as applicable. Spearman Rank correlation analyses were conducted to

identify inter- and intra-kingdom associations, and microbial interactions with AD-related host variables. *P* values <0.05 were considered significant.

RESULTS

A total of 37 swab samples belonging to lesional or non-lesional sites from 10 patients (7 females, 3 males, mean age = 10.5 years, Table 1) were processed for mycobiome analysis. Among the 37 samples collected, nine were from lesional skin, and 10 swabs were obtained from non-lesional skin. One non-lesional sample did not amplify any fungal DNA, and hence was excluded from our dataset. Thus, data from 36 samples were analyzed. Emollient treatment induced a significant reduction of Scoring Atopic Dermatitis (SCORAD) score, from 33.2 ± 5.4 to 9.2 ± 6.4 (Table 1, *P* < .001).

Clustering Analysis. Principal Coordinates Analyses (PCoA) showed that in lesional skin, pre-treatment and post-treatment samples clustered differently at all taxa levels (Fig. 1A-F), although this difference did not statistical significance (*adonis:vegan*, *P* ≥ 0.48). In contrast, in non-lesional samples, no obvious clustering was observed (Fig. 1G-L). Comparison of community distribution using distance to centroid revealed no significant differences between the groups (Supplementary Fig. 1).

Frequency Distribution. Analysis of core mycobiome (taxa with ≥ 20% frequency and >1% abundance) revealed two phyla (Ascomycota, Basidiomycota) to be present in all samples (Fig. 2A), while 10 Class, 16 Order, 19 Families, 13 genera, and 8 species were common in the core biome of all samples (Fig. 2B-F, respectively). Interestingly,

Order Malasseziales was unique to post-treatment samples for both lesional and non-lesional skin (Fig. 2C). At the Genus level, *Coniosporium* was detected only in lesional post-treated samples (Fig. 2E). Common genera across all samples included *Candida*, *Trichosporon*, *Stemphylium*, and *Cladosporium*. Six genera were detected in only non-lesional samples (including *Schizophyllum*, *Perenniporia*, and *Phialocephala*), while four (*Alternaria*, *Coniosporium*, *Debaryomyces*, *Capnodiales*) were detected in only lesional samples.

Diversity. Analysis of the diversity (Shannon Index) of lesional and non-lesional skin mycobiome at different levels of taxa indicated that in non-lesional samples, Shannon diversity index was significantly increased in post-treatment samples at all taxa levels except Phylum (Fig. 3A, $P \leq .04$), while lesional skin exhibited non-significant decrease in diversity after emollient treatment (Fig. 3B). Shannon index values of bacteria were significantly higher than for fungi in both lesional and non-lesional samples ($P \leq .0003$ for all comparisons). There was non-significant increase in richness at different taxa levels in non-lesional skin (Fig. 3C), while a reverse trend was noted in lesional samples (Fig. 3D).

Fungal Abundance. Next, we evaluated the abundance profile of fungi in lesional and non-lesional samples before and after treatment. Ascomycota was the most abundant phylum and Dothideomycetes was the most abundant Class in most samples (Fig. 4). Interestingly, in one subject (#17), pre-treatment samples contained mostly Basidiomycota, while post-treatment samples comprised mostly Ascomycota. Among

both pre-treated and post-treated samples, the main phyla observed in lesional versus non-lesional skin were Ascomycota and Basidiomycota, with no significant difference in abundance between lesional and non-lesional skin ($P \geq 0.1$, data not shown). Moreover, in lesional samples, levels of Ascomycota and Basidiomycota were similar in pre- and post-treatment samples (Figure 5A, $P = 0.269$). However, in non-lesional samples, abundance of Ascomycota tended to increase and that of Basidiomycota tended to decrease for the abundance of Ascomycota and Basidiomycota (Figure 5A, $P = 0.286$, and Supplementary Table 1).

Analysis of other fungal taxa (Class, Order, Family, Genus, and Species) revealed significant difference ($P < .05$) or strong trend ($P < .1$) for several taxa in lesional and non-lesional samples (Figure 5B-D, and Supplementary Tables 2-6). In this regard, abundance of fungal class Dothideomycetes was significantly decreased while that of Saccharomycetes was significantly increased in post-treatment samples (Supplementary Table 2).

Abundance of order Saccharomycetales was increased in post-treatment samples in both lesional and non-lesional skin; this difference was statistically significant in non-lesional samples ($P = .016$, Supplementary Table 3). Abundance of family Gomphidiaceae tended to decrease in post-treatment lesional samples ($P = .07$), while that of Myxotrichaceae was significantly decreased in post-treatment non-lesional samples ($P = .021$, Figure 5B; and Supplementary Table 4). Abundance of family

Sebacinaceae also showed a strong trend to be decreased in post-treatment non-lesional samples compared to pre-treatment non-lesional samples (Fig. 5B, $P = .05$).

Abundance of genus *Chroogomphus* trended to be reduced in post-treatment samples in lesional samples ($P = .07$), while genus *Stemphylium* showed a trend to be reduced in post-treatment non-lesional samples (Fig. 5C, $P = .059$, Supplementary Table 5).

Among both pre-treated and post-treated samples, Genus level comparison showed that *Candida* and *Chroogomphus* were among the most abundant fungi, but their levels did not differ significantly between lesional and non-lesional skin (data not shown).

Levels of Malasseziales, which contains the genus *Malassezia*, were increased by 10-fold in unaffected post-treated skin compared to unaffected pre-treated skin, albeit this difference was not statistically significant (0.30% and 0.03%, respectively; $P \geq 0.3$).

Abundance of *Candida* and *Saccharomyces* spp. were elevated in post-treatment samples for both lesional and non-lesional samples (Supplementary Table 6). A total of eight fungal species were either significantly different ($P < .05$) or showed a strong trend ($P < .1$) between pre- and post-treatment samples of lesional and non-lesional skin (Fig. 5D). These species included *Chroogomphus asiaticus*, *Stemphylium solani*, *Trichosporon debeurmannianum*, uncultured Ascomycota, Basidiomycota, Fusarium, Sebacina, and Oidiodendron (Fig. 5D).

Correlation Analyses

Analysis of correlations among the fungi present at >1% abundance level revealed a strong negative correlation between Ascomycota and Basidiomycota at the phylum level in all samples ($P < .05$ for all comparisons). Moreover, 72 and 114 genera exhibited significant correlations in lesional samples before and after treatment, respectively, while in non-lesional samples, there were 35 and 54 significant genus-level correlations in pre-and post-treatment samples (Supplementary Fig. 2). Analysis of species level correlations identified 108 and 320 significant correlations in pre- and post-treatment lesional samples, while 75 and 136 significant correlations were identified in pre- and post-treatment non-lesional samples (Supplementary Fig. 3).

Post-hoc analyses of inter-kingdom correlations (using bacterial abundance data generated in a previous study⁹) revealed 9 significant positive bacterial-fungal correlations at the Genus level in lesional samples; these sets of correlations were not identical between the treated and untreated groups (Figure 6A,B; $P < .05$ for all comparisons). Among non-lesional samples, 11 and 14 significant inter-kingdom correlations were detected in pre-treated and post-treated groups, respectively (Fig. 6C,D). Analyses of interkingdom correlations at species level revealed 29 and 51 significant positive bacterial-fungal correlations in pre- and post-treatment lesional samples, respectively; while 25 and 37 significant correlations were detected in pre- and post-treatment non-lesional samples (Supplementary Fig. 4).

We also explored the correlations of fungal species with common disease-linked Gram-negative bacteria (*Klebsiella*, *Acinetobacter*, *Pseudomonas*, *Burkholderia* and *Escherichia*) or with Gram positive bacteria (*Corynebacterium*, *Clostridium*, *Listeria*, *Bacillus*, *Staphylococcus*, and *Streptococcus*). In lesional skin, Gram-negative *Pseudomonas* spp. correlated significantly with pathogenic fungal species (*Aspergillus* and *Candida* spp.) in pre-treatment samples; these correlations were not detected in post-treatment samples (Fig. 7A). Moreover, lesional skin exhibited significant correlations between Gram-positive bacteria (*Corynebacterium kroppenstedtii* and *Staphylococcus pettenkoferi*) and *Candida albicans* and *Candida parapsilosis* in pre-treatment samples, but not in post-treated samples (Fig. 7B).

DISCUSSION

Our results demonstrate significant difference (or noticeable trends) between pre- and post-treatment samples obtained from lesional and non-lesional skin, as reflected in the diversity, richness, core mycobiome, and abundance profile of different fungal taxa.

Diversity was significantly increased in treated group in non-lesional skin, which agrees with previous reports associating increase in microbial diversity with health.^{8,9} We also found that abundance of specific fungi changed noticeably between treated and untreated, and also between lesional and non-lesional skin. In this regard, non-lesional skin exhibited changed levels of Ascomycota and Basidiomycota while lesional skin did not exhibit such difference (even though at a clinical level a clear improvement was noticed), suggesting that lesional skin is less prone to Phylum-level changes in

mycobiome in response to application of emollient. However, it is possible that this apparent stability may be indicative of redundancy among the lower taxa members, which compensate for the increase or decrease of specific downstream microbial community members. Such a scenario is very compelling given that other fungal taxa (Class, Order, Family, Genus, and Species) exhibited significant difference or strong trend of changes in abundance in lesional and non-lesional samples.

Our finding that Ascomycota and Basidiomycota were the predominant fungal phyla in tested samples agrees with previous findings. We also found that levels of Malasseziales (which includes the genus *Malassezia*) were increased by 10-fold in post-treated unaffected skin compared to pre-treated skin. Since *Malassezia* is considered a commensal member of a healthy skin mycobiome, these results suggested that treatment with emollient aided in the recovery of the skin mycobiome. However, these abundance levels were very low (< 1%), and the differences did not reach statistical significance. It is possible that the low levels of *Malassezia* in our samples could be because of use of different DNA extraction methods than those used in other studies, or because these samples were not enriched in *Malassezia* due to unrelated environmental or genetic variables. Differences in Gomphidiaceae and *Chroogomphus* are more likely to be transient and related to the environmental variables, than to the pathogenesis of atopic dermatitis. More robust studies need to be conducted to validate these findings and determine their clinical relevance.

Increase in abundance of specific fungi may also be linked to utilization or production of metabolites and biomolecules. In this regard, changes in abundance of fungi belonging to family Sebacinaceae and *Stemphylium* may be linked to utilization of lipids, major components of the skin barrier and defense mechanisms. Interestingly some links with bacteria metabolic pathway could be underlined here. For example, in acne, the relationship between *P. acnes* and *Malassezia* sp. have been already pointed out. Trophic interactions between these bacteria and this polymorphic yeast for lipid catabolism could be a new research target to control those population.

Dysbiosis in skin mycobiome may also influence the cutaneous immune response, with potentially interlinked pathways between specific microbes and the host innate and adaptive immunity. Such interactions have been previously reported between pathogenic (e.g. *Staphylococcus aureus*) and non-pathogenic bacteria and AD-associated inflammation, mediated by specific microbial associated molecular patterns (MAMPs), interleukin-10 expression and regulatory T cell phenotypes.²¹

Lesional and non-lesional skin exhibited distinct inter-kingdom correlations between bacteria and fungi in pre- and post-treated skin samples. While interesting trends were noted at phylum level (e.g. between Firmicutes/Fusobacteria with Ascomycota and Basidiomycota), several of these correlations reached statistical significance at the genus and species levels. Particularly intriguing was the observation that in pre-treated samples, positive correlations were detected among bacteria and *Aspergillus*, a known fungal pathogen; these correlations were not detected in post-treated samples.

Moreover, positive correlations between *Pichia* (a fungus with beneficial properties for human health) and bacteria (e.g. *Demacoccus*) were detected in emollient-treated samples. *Pichia* is a known biocontrol agent, and has been recommended by the European Food Safety Authority (EFSA) for prevention of pathogenic organism in food products.²² Moreover, the observation that both Gram-negative and -positive bacteria were significantly correlated with pathogenic fungi (*Aspergillus* spp., *Candida* spp.) in pre-treatment samples of lesional skin but not in post-treatment skin suggested that treatment with emollient may encourage growth and interactions between beneficial microbes on skin, while also suppressing the growth of harmful microbes.

Potential caveats of our study are the low sample size, post-hoc nature of the inter-kingdom analyses, and lack of in vitro and in vivo validation. Therefore, the clinical relevance of our results remains to be investigated. Further preclinical and clinical studies with robust sample size are warranted to validate the interactions observed in the current pilot study, and to determine their functional significance. Nevertheless, these results generate several testable hypotheses, and will guide the design and conduct of future studies.

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Table 1. Demographics summary of enrolled patients

Patient ID	Body Site	Gender	Age (yrs)	SCORAD*	SCORAD	Δ SCORAD	Δ SCORAD %
				(Day 0)	(Day 84)		
1	Elbow	Female	5	26.6	0	-26.6	-100%
2	Palm	Female	6	36.6	8.4	-28.2	-77%
3	Elbow	Female	3	33.6	13.1	-20.5	-61%
10	Finger	Female	19	39.1	23.4	-15.7	-40%
13	Elbow	Male	14	33.1	7.8	-25.3	-76%
14	Elbow	Male	5	25.1	5.7	-19.4	-77%
15	Finger	Female	17	39.8	4.7	-35.1	-88%
17	Finger	Female	24	38.6	9.3	-29.3	-76%
22	Arm	Female	3	33.6	6.1	-27.5	-82%
26	Cheek	Male	9	26.6	13.7	-12.9	-48%

*SCORAD: "Scoring atopic dermatitis", a severity score used for atopic dermatitis.

Δ SCORAD: reduction in SCORAD on Day 84 compared to Day 0. Δ SCORAD %:

reduction in SCORAD as a percentage of the score on Day 0.

FIGURE LEGENDS

Figure 1. Principal coordinates analysis (PCoA) of abundance of fungal taxa in (A-F) lesional and (G-L) non-lesional samples, before and after treatment with emollient. Clustering at all taxa levels (Phylum, Class, Order, Family, Genus, Species) are shown. Ellipses are drawn at a confidence level of 0.95. Principal coordinates analysis (PCoA) was conducted with identified reads/OTUs using classical multidimensional scaling (functions *dist/euclidean*, *cmdscale*) to analyse distribution of dissimilarities and hierarchical clustering in dendrograms in *vegan*.²⁴

Figure 2. Effect of emollient treatment on the core mycobiome lesional and non-lesional skin in AD patients. Numbers indicate the actual count of members identified in each taxon (A-E: Phylum through Species). Core biome comprised taxa with frequency of at least 20% (and relative abundance $\geq 1\%$). LPre: Lesional Pre-Treatment, LPost: Lesional Post-Treatment, nLPre: non-Lesional Pre-Treatment, nLPost: non-Lesional Post-Treatment samples.

Figure 3. Diversity (Shannon index and richness) profile of non-lesional and lesional samples, compared between pre-treatment and post-treatment groups at different taxa levels. * $P \leq .04$, for comparison between treated and untreated groups.

Figure 4. Stack bar charts showing sample-wise distribution of fungal (A) Phylum and (B) Class, in non-lesional and lesional samples, before and after treatment

with emollient. Data for Phylum or Class only present at proportion ≤ 0.01 (abundance $\leq 1\%$) are shown.

Figure 5. Summary abundance of fungal taxa in lesional and non-lesional skin in pre-treatment and post-treatment samples. (A) Phylum, (B) Family, (C) Genus, and (D) Species. P-values represent comparisons between Pre- and Post-Treatment samples.

Figure 6. Inter-kingdom correlations between fungal and bacterial genera in lesional and non-lesional samples of atopic dermatitis (pre- and post-treatment). Statistically significant correlations (Spearman) are shown ($P < .05$).

Figure 7. Effect of emollient treatment on inter-kingdom correlations between fungal species and selected (A) Gram-negative and (B) Gram-positive bacterial species. Statistically significant ($P < .05$) Spearman correlations are shown.

Supplementary Figure 1. Homogeneity of multivariate dispersions in tested groups. Differences in group homogeneities were determined by calculating the distance to centroid for each group (*betadisper* function in *vegan*) at different taxa: (A) Class, (B) Order, (C) Family, (D) Genus, and (E) Species

Supplementary Figure 2. Intra-kingdom correlations among fungal genera in pre- and post-treatment samples of lesional and non-lesional skin. Statistically

significant Spearman correlations are shown ($P < .05$). Red: negative correlations; blue: positive correlations.

Supplementary Figure 3. Intra-kingdom correlations among fungal species in pre- and post-treatment samples of lesional and non-lesional skin. Statistically significant Spearman correlations are shown ($P < .05$). Red: negative correlations; blue: positive correlations.

Supplementary Figure 4. Inter-kingdom correlations between fungal and bacterial species in pre- and post-treatment samples of lesional and non-lesional skin. Statistically significant Spearman correlations are shown ($P < .05$). Red: negative correlations; blue: positive correlations.