Shifts in the Skin Microbiota after UVB Treatment in Adult Atopic Dermatitis

Astrid Haaskjold Lossius[a, b, c]   Olav Sundnes[a, b, c]   Anna Cäcilia Ingham[d]

Sofie Marie Edslev[d]   Jørgen Vildershøj Bjørnholt[a, e]   Berit Lilje[d]

Maria Bradley[f]   Samina Asad[f]   Guttorm Haraldsen[a, c]   Paal Skytt-Andersen[d]

Jan-Øivind Holm[a, b]   Teresa Løvold Berents[b, g]

[a]Institute of Clinical Medicine, University of Oslo, Oslo, Norway; [b]Department of Dermatology, Oslo University Hospital, Oslo, Norway; [c]Department of Pathology, Oslo University Hospital, Oslo, Norway; [d]Department of Bacteria, Parasites, and Fungi, Statens Serum Institut, Copenhagen, Denmark; [e]Department of Microbiology, Oslo University Hospital, Oslo, Norway; [f]Division of Dermatology and Venereology, Department of Medicine, Karolinska Institutet, Stockholm, Sweden; [g]Regional Unit of Asthma, Allergy and Hypersensitivity, Oslo University Hospital, Oslo, Norway

Abstract

Background: The pathophysiology in atopic dermatitis (AD) is not fully understood, but immune dysfunction, skin barrier defects, and alterations of the skin microbiota are thought to play important roles. AD skin is frequently colonized with *Staphylococcus aureus* (*S. aureus*) and microbial diversity on lesional skin (LS) is reduced compared to on healthy skin. Treatment with narrow-band ultraviolet B (nb-UVB) leads to clinical improvement of the eczema and reduced abundance of *S. aureus*. However, in-depth knowledge of the temporal dynamics of the skin microbiota in AD in response to nb-UVB treatment is lacking and could provide important clues to decipher whether the microbial changes are primary drivers of the disease, or secondary to the inflammatory process. ***Objectives:*** To map the temporal shifts in the microbiota of the skin, nose, and throat in adult AD patients after nb-UVB treatment. ***Methods:*** Skin swabs were taken from lesional AD skin (*n* = 16) before and after 3 treatments of nb-UVB, and after 6–8 weeks of full-body treatment. We also obtained samples from non-lesional skin (NLS) and from the nose and throat. All samples were characterized by 16S rRNA gene sequencing. ***Results:*** We observed shifts towards higher diversity in the microbiota of lesional AD skin after 6–8 weeks of treatment, while the microbiota of NLS and of the nose/throat remained unchanged. After only 3 treatments with nb-UVB, there were no significant changes in the microbiota. ***Conclusion:*** Nb-UVB induces changes in the skin microbiota towards higher diversity, but the microbiota of the nose and throat are not altered.

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Introduction

Atopic dermatitis (AD) is the most prevalent inflammatory skin disease, with a broad impact on patients' quality of life and on the health care system [1, 2]. It is characterized by itchy, red, scaly skin and increased risk

Correspondence to:
Astrid Haaskjold Lossius, astridha@medisin.uio.no

of skin infections [3]. The pathogenesis is complex, and immune dysfunction, skin barrier defects, and alterations in the skin microbiota are thought to play important roles [2, 4, 5]. Whether the microbial abnormalities are primary drivers of AD or whether they are secondary events resulting from inflammation or skin barrier dysfunction is still not fully elucidated.

Colonization of the skin with *Staphylococcus aureus* (*S. aureus*) is more frequent in patients with AD [6, 7], and the abundance of *S. aureus* correlates with disease severity [7–9]. This is partly explained by *S. aureus* thriving better in the higher pH conditions of AD skin than in the more acidic environment of healthy skin [10]. In AD skin, there is an increased relative abundance of *S. aureus* and *S. epidermidis*, while on the genus level, *Cutibacterium*, *Streptococcus*, *Acinetobacter*, *Corynebacterium*, and *Prevotella* are decreased [11, 12].

During eczema flares, the lesional skin (LS) microbiota becomes less diverse, but after weeks of adequate therapy, it seems to approximate diversity levels comparable to baseline [13–19]. A recent review emphasized the inconsistent findings on bacterial diversity on LS versus non-lesional skin (NLS) in AD [20]. While most studies report lower diversity on LS than NLS [12, 21, 22], some report no differences [23, 24].

Interestingly, the nasal microbiota in AD patients is more uniform than in healthy controls [23], and the microbial composition in the nose correlates to AD severity in children with increased abundance of *Staphylococcus* spp. in more severe AD [25, 26].

Phototherapy with narrow-band ultraviolet B (nb-UVB) exerts anti-bacterial effects [27–29] and alters the levels of the anti-microbial peptides (AMPs) defensins, S100-proteins, cathelicidin, and ribonuclease 7 [30–32]. Previous studies have shown, by culture-based methods, that UVB treatment decreases the relative abundance of *S. aureus* in AD skin in adults and children [33–35]. However, conventional culturing fails to grow 80% of bacterial species, and applying culture-independent methods like 16S rRNA gene sequencing means the whole bacterial community can be characterized [36, 37].

We aimed to map the changes in the skin, nose, and throat microbiota with 16S rRNA gene sequencing before and after 6–8 weeks of full-body nb-UVB treatment in adult patients with AD. We also wanted to explore whether only 3 treatments of nb-UVB could induce any shifts in the skin microbiota before signs of clinical remission were evident.

Materials and Methods

Patients

Adults (*n* = 16) with AD, according to the criteria of Hanifin and Rajka [38], were recruited to the study and the same cohort has been described previously [39]. Prior to inclusion, systemic antibiotics and immunosuppressive therapy were avoided for 4 weeks. Topical immunosuppressive therapy (corticosteroids and calcineurin inhibitors) and topical anti-bacterial therapy were avoided for 2 weeks. Structured interviews were performed and disease severity and morbidity were assessed with validated scoring tools: the Eczema Area and Severity Index (EASI) [40], the SCORing Atopic Dermatitis (SCORAD) index [41], the Patient-Oriented Eczema Measure (POEM) [42], and the Dermatology Life Quality Index (DLQI) [43]. Saliva was collected in Oragene™ DNA saliva sampling kits (DNA Genotek, Ottawa, ON, Canada) and tested for the 3 most common mutations in the genes encoding filaggrin (*FLG*): R501X, R2447X, and 2282del4. Serum samples, to measure IgE, eosinophils, and vitamin D levels, and microbial samples were collected.

Intervention

The nb-UVB minimal erythema dose [44] was established by Dermalight® 80 MED-tester (Dr Hönle, Medizintechnik, Gilching, Germany) and 1 lesion with active AD was chosen, primarily the antecubital crease. This lesion was irradiated with 1 MED on days 0, 2, and 4 with the small, hand-held nb-UVB device, Dermalight® 80. Subsequently, the patients underwent full-body nb-UVB 3 times a week for 6–8 weeks, with incremental dosages, with a total of 12–25 treatments. Clinical severity scores (EASI, SCORAD, POEM, and DLQI) were registered before treatment (day 0), after 3 treatments (day 7), and after 6–8 weeks of treatment.

Microbial Sampling

Microbial samples were collected at 3 time points: on days 0 and 7 (before and after local short-term nb-UVB treatment on days 0, 2, and 4) and after full-body treatment (3 times/week for 6–8 weeks). Samples were collected by rubbing a dry ESwab (Copan, Brescia, Italy) against an area of 5 cm² of LS and NLS for 30 s. LS samples were primarily taken from the antecubital crease, while NLS skin was sampled from the nates, regarded as UV-naïve skin before treatment. Nose samples were obtained by rotating a dry Eswab in both anterior nares and the throat samples by rubbing the fauces. All samples were immediately frozen and stored at −80°C.

DNA Extraction and Amplicon Sequencing

Bacterial DNA was isolated from Eswab after enzymatic prelysis by mixing 200 μL sample with 50 μL enzymatic TE lysis buffer (lysostaphine [SAE0091] 2,5U, mutanolysin [sae0092] 25U, lysozyme [L4919] 3 mg [Sigma-Aldrich, St. Louis, USA]) for 30 min at 37°C, adding 20 μL proteinase K (RPROTKSOL-RO, Sigma-Aldrich), and incubated at 56°C for 30 min. 200 μL was extracted on a MagNA Pure 96 system (Roche, Mannheim, Germany) with a DNA and Viral NA small volume kit (Roche). Previously evaluated primers (341F: 5′-CCTACGGGNGGCWGCAG-3′; 805R: 5′-GACTACHVGGGTATCTAATCC-3′), preceded by heterogeneity spacers, were used for amplification of the V3-V4 region of the 16S rRNA gene [45]. Library construction and sequencing was

performed on Illumina MiSeq (Illumina Inc., San Diego, USA), using a 600 cycle V3 kit. To resolve species-level affiliations within the genus *Staphylococcus*, we implemented a *tuf*-gene sequencing approach as previously described [46].

Pre-Processing of Sequencing Data

Raw reads were demultiplexed using the *bcl2fastq* conversion software (Illumina, San Diego, CA, USA). Heterogeneity spacers and primers were trimmed off at an 8% error rate (1 mismatch per primer sequence) with cutadapt (v2.3) of 16S rRNA and *tuf* gene reads [47]. Both primers had to be detected in the respective reads to retain a read pair. We used the *R* package dada2 (v1.12.1) for amplicon sequence variant (ASV) inference from trimmed reads [48]. Except for the truncation lengths (Appendix 1), the dada2 pipeline was used with default settings. Consensus removal of chimeras was performed. Samples with a read count < 5,000 after quality filtering were re-sequenced. ASVs resulting from 16S rRNA gene sequences were taxonomically classified with dada2's *assignTaxonomy()* and *addSpecies()* functions, using the Silva reference database and species-level training set (v132), respectively [49]. Staphylococcal ASVs from *tuf* gene sequences were classified with the *assignTaxonomy()* function, based on the taxonomic database designed by Iversen et al. [46].

The ASV count tables and taxonomic table were integrated using the *R* package phyloseq [50]. The 16S rRNA gene-derived count data of skin, nose, and throat samples was separately subjected to contaminant identification removal using the *R* package decontam [51] and manually contaminant removal (Appendix 2). One skin sample with < 4,500 reads after merging was excluded from downstream analysis.

Statistical Analysis

Statistical analyses were performed in *R* v3.6.0 [52] with the packages phyloseq and its dependencies, vegan, cluster, factoextra, markovchain, and lmerTest [50, 53–58]. Visualizations were generated with ggplot2, diagram, and ComplexHeatmap [59–61]. For each sample, bacterial alpha diversity, the diversity within samples, and measurements of both species' richness and evenness, were calculated on raw counts by the inverse Simpson index. Alpha diversity on LS and NLS before treatment was compared using a paired Wilcoxon signed-rank test. Alpha diversity before treatment, and after local short-term and full-body treatment for 6–8 weeks was compared by paired Wilcoxon signed-rank tests. Correlation between the number of nb-UVB sessions and change in bacterial diversity was calculated by Spearman's rank correlation. All presented taxonomic barplots are based on relative abundance.

The count data was Hellinger-transformed, i.e., sample-wise proportions were calculated, and subsequently square root-transformed. Principal co-ordinates analysis (PCoA) based on the Bray-Curtis distance was performed to visualize differences in the bacterial community structure over time. Samples were grouped into community state types (CSTs) by partitioning around medoid (PAM) clustering, based on the Jensen-Shannon distance. A consensus decision about pre-determining the optimal number of clusters was made by means of the gap statistic, silhouette width, and the elbow method. CST dynamics were visualized as Markov chains showing transition probabilities between CSTs over time.

We performed canonical correspondence analysis (CCpnA), a multivariate constrained ordination method, to model bidirec-

tional relations between ASV abundances and CST affiliation, alpha diversity, and disease severity. The function *cca()* from the package vegan [53] was used.

The 16S rRNA gene and *tuf* gene sequences are available through the European Nucleotide Archive (ENA) at the European Bioinformatics Institute (EBI) under accession No. PRJEB41859.

Table 1. Adults with atopic dermatitis (*n* = 16)

Median age, years (range)	25.5 (20–73)
Sex	
Female	11
Male	5
Filaggrin mutation[a]	
WT	12
Mutation	4
Serum IgE[b]	
Elevated	8
Normal	8
Eosinophil count[c]	
Elevated	2
Normal	10
Not available	4
Vitamin D supplementation	
Yes	9
No	7
Median number of nb-UVB sessions (range)	20 (12–25)

Values express *n*, unless otherwise indicated. Serum was sampled from all patients prior to nb-UVB treatment for the measurement of IgE and eosinophils. nb-UVB, narrow-band ultraviolet B; WT, wild type.

[a] Saliva was genotyped for the most common mutations in the genes encoding filaggrin: R501X, R2447X, and 2282del4. Patients with the R501X mutation were heterozygous; 1 patient was compound heterozygous (2 different mutations: R501X/R2447X).

[b] Reference range 0–114 kU/L.

[c] Reference range $<0.4 \times 10^9$/L.

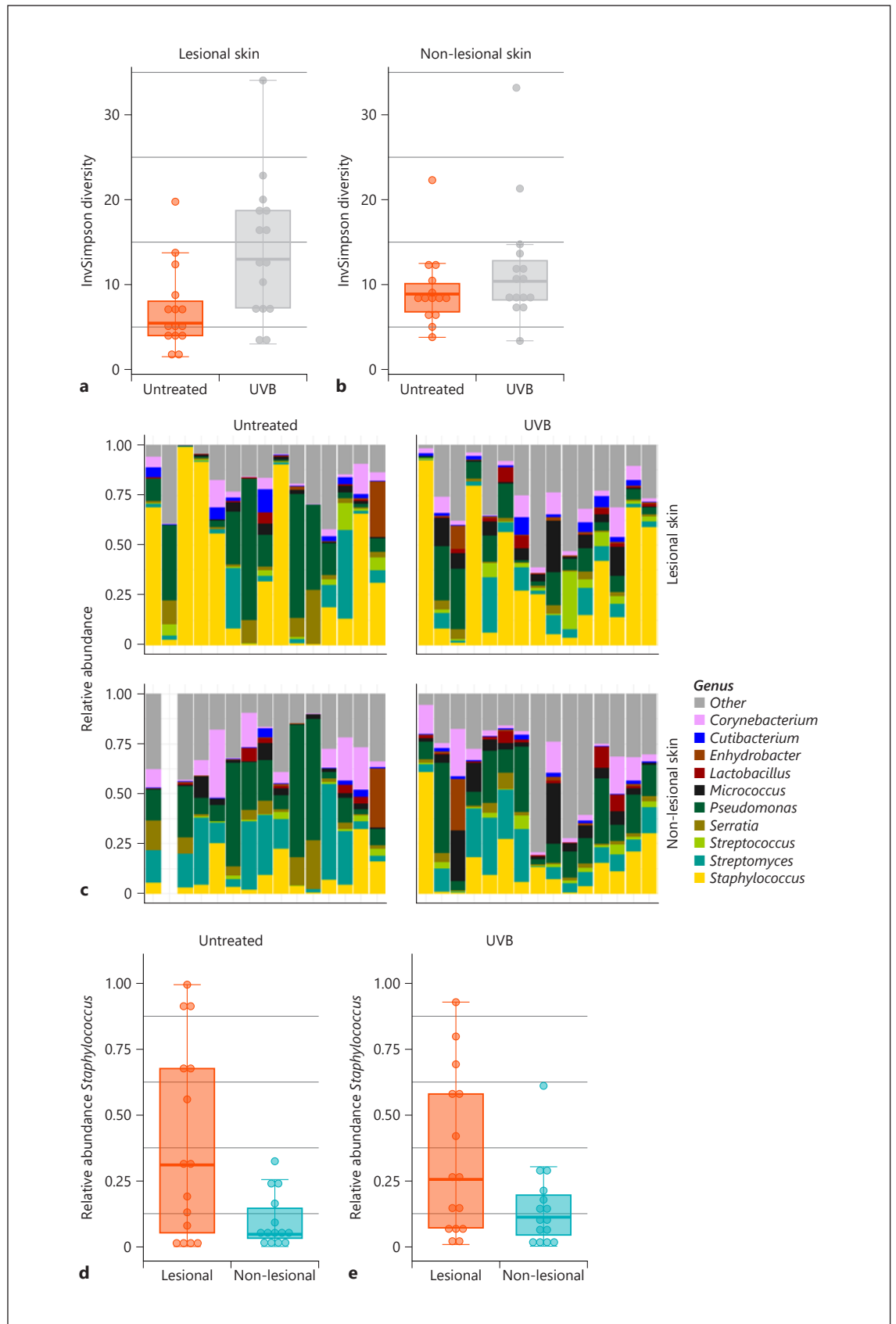**Table 2.** Vitamin D levels and clinical severity scores before and after treatment

	Before treatment	After treatment
Serum vitamin D[a] levels	44–116 (81.1)	67–174 (123.4)
EASI	3.6–14.7 (9.1)	0–24.3 (5.1)
SCORAD	22.5–65 (40.8)	5–69.5 (27.2)
POEM	8–23 (16.6)	4–23 (10.6)
DLQI	5–22 (11.2)	1–11 (5.3)

Values express range (mean). EASI, Eczema Activity and Severity Index; SCORAD, SCORing Atopic Dermatitis index; POEM, Patient-Oriented Eczema Measure; DLQI, Dermatology Life Quality Index.

[a] 25-OH-vitamin D (reference range 37–131 nmol/L).

(For legend see next page.)

Results

Adults with AD (n = 16) were included (11 females and 5 males; median age 25.5 years [range 20–73 years]) and clinical and demographic data are presented in Table 1. Four of the patients had a mutation in the gene encoding *FLG* (Table 1). At inclusion, most patients scored a moderate AD (Table 2), and after 6–8 weeks of treatment there was a reduction in all the severity scores in most patients as previously described [39]. Vitamin D levels increased significantly (Table 2; online suppl. Fig. S1; see www.karger.com/doi/10.1159/000515236 for all online suppl. material).

Bacterial Diversity Increases on LS after 6–8 Weeks of nb-UVB

The bacterial alpha diversity increased on LS after 6–8 weeks of treatment with nb-UVB (p = 0.013; Fig. 1a). In contrast, the difference before and after treatment on NLS was not significant (Fig. 1b). We observed a trend of lower alpha diversity on LS than on NLS before treatment, although not significant (data not shown). There was no correlation between the number of nb-UVB sessions and change in bacterial diversity for LS (Spearman's rank correlation ρ: 0.1320122, p = 0.65) or NLS (Spearman's rank correlation ρ: –0.2702212, p = 0.37; data not shown).

The relative proportions of the different genera on LS compared to NLS before and after treatment are shown in Figure 1c; the plot shows greater abundance of *Staphylococcus* on LS than on NLS before and after treatment. The relative abundance of *Staphylococcus* was higher on LS than on NLS before treatment (p = 0.001, Fig. 1d). After treatment for 6–8 weeks, the relative abundance of *Staphylococcus* was still higher on LS (p = 0.001, Fig. 1e). We further did *tuf*-sequencing to explore whether the relative abundances of *S. aureus* and *S. epidermidis* changed after treatment, but we did not observe any significant changes after 6–8 weeks of treatment with nb-UVB (data not shown). Also, we did not find any significant changes in the abundance of Pseudomonadaceae (data not shown).

Dynamics of the Bacterial Communities

To investigate the dynamics of the bacterial community, we did clustering into CSTs, and 4 different CSTs were identified. CST 1 and CST 4 were mainly dominated by the family Staphylococcaceae. At the genus level, CST 1 and CST 4 were dominated by *Staphylococcus*, while CST 3 was dominated by *Pseudomonas* (Fig. 2). We implemented a CCpnA to model multivariate relationships between CST affiliation, alpha diversity, and clinical severity scores, which visually confirmed the clustering of the samples into 4 CSTs (Fig. 3). CSTs 1 and 4 were more
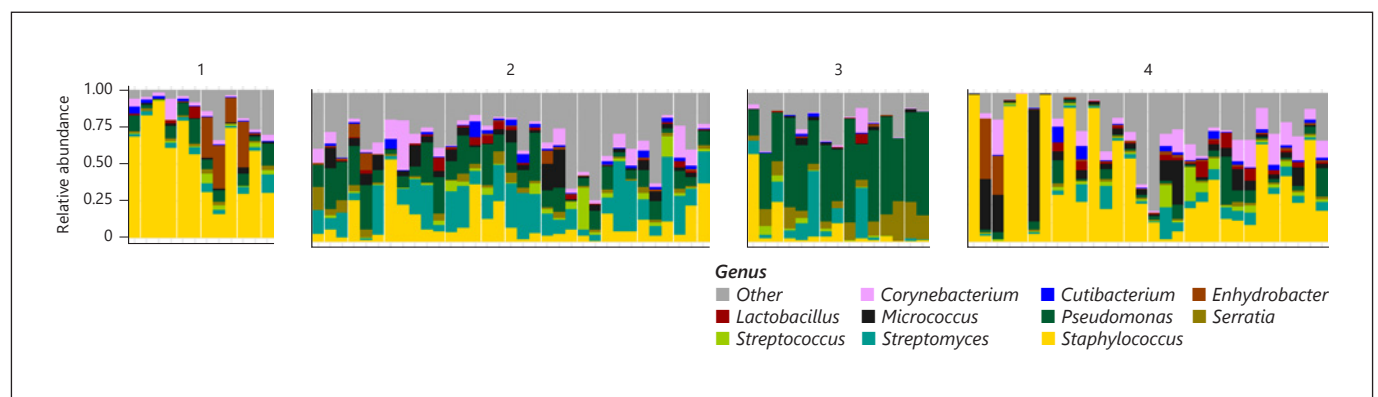

Fig. 2. Community state types (CSTs) in adults with atopic dermatitis. Four different CSTs were identified. At the genus level, CST 1 and CST 4 were dominated by *Staphylococcus*, while CST 3 was dominated by *Pseudomonas*. Each column represents 1 sample.

Fig. 1. Bacterial alpha diversity increases on lesional skin after nb-UVB treatment for 6–8 weeks in adults with atopic dermatitis. InvSimpson diversity before and after treatment on lesional skin (LS; p = 0.013; **a**) and non-lesional skin (NLS; p = 0.305; **b**). **c** Proportions of predominant genera on LS and NLS before and after nb-UVB treatment: *Staphylococcus* (in yellow). Each column represents 1 sample. The relative abundance of *Staphylococcus* was higher on LS than NLS for untreated (p = 0.001; **d**) and treated skin (p = 0.001; **e**).
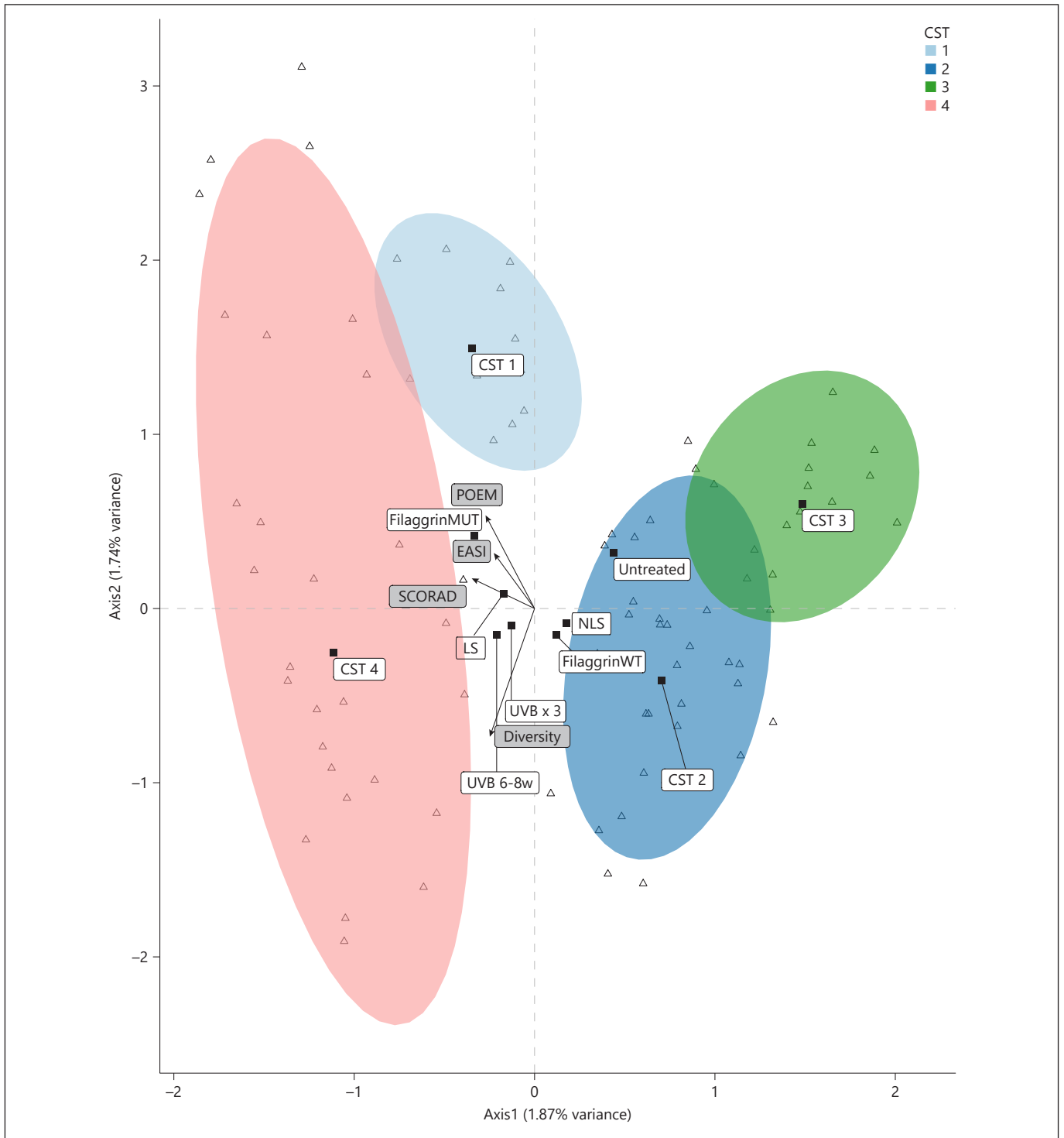
Fig. 3. Canonical correspondence analysis (CCpnA) to model the relationship between community state type (CST) affiliation, bacterial alpha diversity, and clinical severity in adults with atopic dermatitis. The colored ellipses represent the CSTs and the samples cluster into 4 distinct CSTs. CSTs 1 and 4 are more common in lesional skin (LS) than in non-lesional skin (NLS). Clinical severity scores are higher in samples grouped into CSTs 1 and 4, and lower in samples grouped into CST 2. Black squares represent centroids of categorical variables (white labels). Arrows represent continuous variables (grey labels). Triangles represent samples. UVB, ultraviolet B; FilaggrinMUT/WT, filaggrin mutation present/wild type; EASI, Eczema Activity and Severity Index; SCORAD, Scoring Atopic Dermatitis index; POEM, Patient-Oriented Eczema Measurement.

common in LS than in NLS samples. Clinical severity scores were higher in samples grouped into CSTs 1 and 4 and lower in samples grouped into CST 2 (Fig. 3).

Clinical severity scores were higher when an *FLG* mutation was present (Fig. 3) but due to the small number of patients with a mutation, this association remains inconclusive and must be confirmed in a larger study population. Transition probabilities between CSTs were visualized as Markov chains (online suppl. Fig. S2). CSTs 1 and 4 were the most stable, while CST 2 was more dynamic. Although not statistically significant, we observed a trend towards decreasing clinical severity scores when shifting to CST 2 after nb-UVB treatment compared to switching to or staying in another CST (data not shown).

Nose and Throat Microbiota Are Not Affected by Full-Body nb-UVB Treatment

We could not find any changes in alpha diversity in the nose or throat after 6–8 weeks of full-body treatment with nb-UVB (data not shown). The relative abundance on the genus level in the nose is shown in Figure 4a; according to the plots, there were no major alterations before and after treatment. We also tested if the relative abundance of the genus *Staphylococcus* was altered, but it did not change (Fig. 4b). We could not observe any major changes in the relative abundance on the genus level in the throat either (Fig. 4c).

Short-Term Treatment Does Not Result in Observable Microbiota Shifts

To shed light on whether nb-UVB could induce early shifts in the skin microbiota before any signs of clinical remission were evident, we looked at alpha diversity on LS before and after only 3 treatments of local nb-UVB. We did not observe any statistically significant changes in microbial alpha diversity (online suppl. Fig. S3) and the relative abundance of the genus *Staphylococcus* was not changed (data not shown). Plots of relative abundances on the family or genus level did not reveal any major alterations (data not shown).

Discussion

In this study, we show that full-body nb-UVB treatment for 6–8 weeks changes the composition of the microbiota on LS, but not on NLS, in AD. We have previously shown that gene expression changes are evident in AD skin after only 3 treatments of nb-UVB; specifically, we found altered gene expression of several AMPs and

transcripts related to inflammation, epidermal structure, and keratinization [39]. In contrast, we found no significant changes in the skin microbiota after 3 treatments of nb-UVB. Taken together, these findings may suggest that the changes in the microbiota are secondary to skin inflammation and not a primary driver of AD pathogenesis, a question which has been raised by several authors [2, 4, 5].

Our findings of diversity change during treatment are in line with previous studies where alpha diversity increased with clinical improvement [15, 16, 62]. Conventional culturing methods have shown that UVB re-establishes the skin microbiota in AD [33–35], but this is the first study using 16S rRNA gene sequencing to show that nb-UVB alone induced changes in the microbiota. A previous study showed increased alpha diversity after 4–6 weeks of treatment with nb-UVB combined with topical corticosteroids [62], but no significant difference from treatment with corticosteroids alone, implying no additional effect of nb-UVB. In the group treated with combination therapy, however, clinical severity scores (EASI) decreased further 3 weeks after discontinuing treatment, indicating a possible positive long-term effect of nb-UVB [62].

Topical corticosteroid treatment for 4 weeks has also been shown to increase the alpha diversity in children with AD [15], but a shorter treatment duration (7–10 days) did not have any effect on bacterial diversity in infants [63]. A study on dupilumab (a monoclonal antibody blocking interleukin [IL]-4 and IL-13) in adult AD patients showed increased alpha diversity and a lower abundance of *S. aureus* on LS and NLS after 16 weeks of treatment, but the effect did not last; 18 weeks after discontinuation of treatment, the microbiota closely resembled the pre-treatment state [21].

The effect of UVB is not restricted to AD, as a recent study in healthy subjects ($n = 6$) showed that both UVA and UVB influence the composition of the skin microbiota [44]. In this study on healthy skin, the relative abundance of Pseudomonadaceae decreased after nb-UVB exposure [44], but we could not replicate this finding in our material. This could possibly reflect the differences in the skin microbiota in healthy and atopic skin. In psoriasis, another chronic inflammatory skin disease, nb-UVB treatment induces no significant alterations in alpha or beta diversity but significant changes on the genus level [64].

The nasal microbiota in patients with AD is less diverse than in healthy controls [23]. We hypothesized that full-body nb-UVB treatment might also modulate the na-
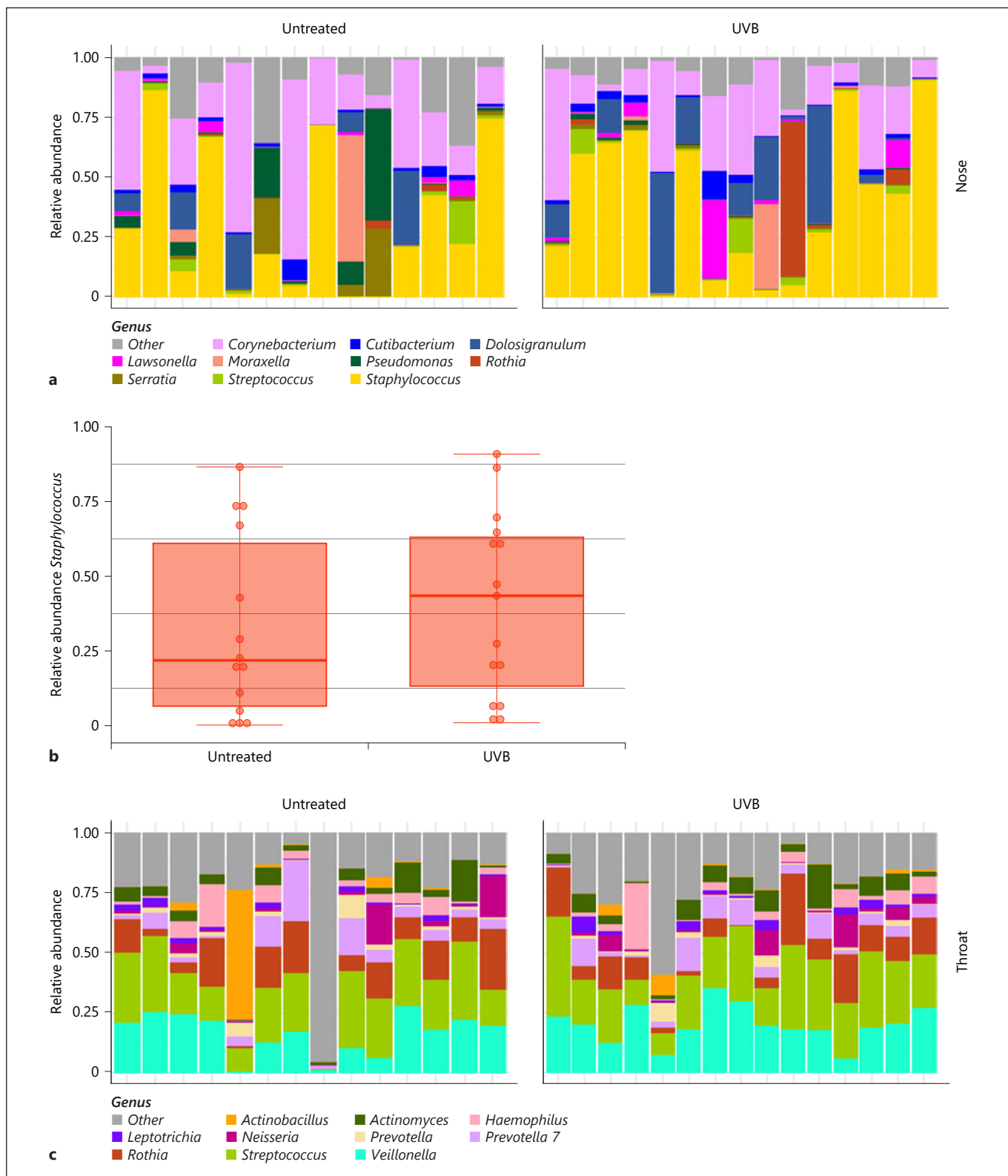
Fig. 4. The microbiome of the nose and throat in adults with atopic dermatitis. **a** In the nose, there were no changes on the genus level after treatment. **b** The relative abundance of the genus *Staphylococcus* did not change. **c** In the throat, there were no changes after treatment on the genus level. Each column represents 1 sample.

sal and throat microbiota. However, we found no changes in alpha diversity in the nose or throat after 6–8 weeks treatment with nb-UVB, suggesting that UVB irradiation of the skin has less effect on the microbiota on mucosal surfaces. We are not aware of any studies having explored the effect of nb-UVB on the nasal or throat microbiota in AD. However, a recent study showed that nb-UVB irradiation of the skin in healthy individuals is associated with increased diversity of the gut microbiota [65]. This modulation was restricted to individuals not on vitamin D supplements, suggesting that vitamin D levels could influence the microbial composition of the gut. Limited UVB exposure and low vitamin D is thought to partly explain the prevalence of several other chronic inflammatory diseases and the correlation between latitude and disease severity [66, 67]. In our study, half of the participants took some form of vitamin D supplements (Table 1), but we did not stratify on this parameter in our analyses. Serum vitamin D levels increased in our cohort, confirming previous reports [68–70].

Our patient cohort is well-characterized, and disease severity is scored by validated scoring tools in line with guidelines from the Harmonizing Outcome Measuring Eczema (HOME) initiative, in order to make different studies easier to compare [71]. The patient population seems quite representative of adult AD patients; approximately 25% had an *FLG* mutation which corresponds to previously reported numbers [72]. Most of the patients in our cohort responded well to treatment with nb-UVB [39], consistent with previous reports on nb-UVB treatment [73, 74].

The main limitation of our study is the small sample size, which rendered it mainly explorative; further studies with more participants are needed to validate our results. Although16S rRNA gene sequencing provides extensive information about microbial communities on the skin [37], the copy number of the 16S rRNA gene varies between bacterial species and may lead to an over-representation of some species [75]. Furthermore, 16S rRNA gene sequencing results are compositional rather than absolute, and our analysis did not determine any cause-and-effect relationships [36]. Taxonomy is dependent on the reference database used [36] and technical aspects like sampling technique, DNA extraction, and sequencing protocol may introduce some degree of uncertainty, especially between studies [76, 77]. To introduce as little variation as possible, all samples in this study were processed and treated the same way.

Despite the obvious dysbiosis in the eczematous skin of patients with AD, treatments modulating the microbiota have not been central in clinical practice. Two small-scale trials reintroduced commensal skin bacteria in human subjects with AD. Topical application of coagulase-negative *Staphylococcus* spp. decreased the colonization by *S. aureus* [78], and topical application of the Gram-negative *Roseomonas mucosa* led to clinical improvement [79]. More knowledge regarding the complex interplay between the skin microbiota and the immune system could pave the way for more individualized treatment.

Treatment with nb-UVB for 6–8 weeks in adults with AD induced shifts in the skin microbiota in LS, but did not affect the microbiota of NLS, or the nose and throat. The changes in LS appeared after clinical remission was evident, suggesting that the microbial changes are secondary, rather than primary, in the pathogenesis of AD.

Key Message

UVB treatment for 6–8 weeks increases bacterial diversity in lesional skin in atopic dermatitis.

Appendix 1

Truncation Length Adjustments
For 16S rRNA reads, truncation lengths were adjusted to 270 bp for forward reads and 210 bp for reverse reads. For *tuf* gene reads, forward reads were truncated at 270 bp and reverse reads were truncated at 241 bp.

Appendix 2

Contaminant Removal
Contaminants were identified and removed manually: ASVs classified no further than class-level and belonging to either the phyla *Cyanobacteria*, *Plantomycetes*, *Chloroflexi*, and *Deinococcus-Thermus*, or the orders Rhizobiales, Rhodobacterales, and Oceanospirillales. From the skin sample data set, 38 ASVs were identified as contaminants and removed (decontam method "either", frequency threshold 0.1, prevalence threshold 0.25). In addition, 224 ASVs were removed manually. From the nasal data set, we removed 27 ASVs (decontam method "either", frequency threshold 0.05, prevalence threshold 0.25), and 89 ASVs were removed manually. We removed 47 ASVs from the throat data set (decontam method "either", frequency threshold 0.1, prevalence threshold 0.25). Twenty-five ASVs were manually removed. After contaminant removal, read counts of re-sequenced samples were merged.

Acknowledgements

Statement of Ethics

The Regional Ethics Committee approved the study (2017/466). All participants gave informed written consent.

Conflict of Interest Statement

AHL has received an unrestricted research grant from Sanofi.

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Author Contributions

A.H.L., T.L.B., J.-Ø.H., O.S., and G.H. designed the study. A.H.L. included the patients. J.V.B. handled the samples. S.M.E., A.C.I., B.L., and P.S.-A. sequenced and analyzed the samples. S.A. and M.B. did the filaggrin analyses. A.H.L. drafted the manuscript and all co-authors contributed to the editing process.

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