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The nasal and skin microbiome are associated with disease severity in pediatric atopic dermatitis

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ABSTRACT

Background

Changes in the skin microbiome have been associated with atopic dermatitis (AD) and its severity. The role of the nasal microbiome in relation to the severity of AD and its relation with the skin microbiome, are less well studied.

Objective

We aimed to characterize the nasal and skin microbiome in children with AD in relation to disease severity. Additionally, we explored differences and correlations between the nasal and skin communities.

Methods

We characterized the microbial composition of nasal and lesional skin samples from 90 and 108 patients with AD, respectively, using 16S-rRNA sequencing. Additional quantitative (q)PCR for *Staphylococcus (S.) aureus* and *S. epidermidis* was performed on the skin samples. Disease severity was estimated using the self-administered eczema area and severity index.

Results

We found an association between the microbial composition and AD severity in both the nose and skin samples (R^2=2.6%; p=0.017 and R^2=7.0%; p=0.004). Staphylococci were strong drivers for the associations with severity. However, other species also contributed, such as *Moraxella* in the nose. Skin lesions were positive for *S. aureus* in 50% of the children and the presence and load of *S. aureus* was not associated with disease severity. Although the nose and skin harbor distinct microbial communities based on Bray–Curtis dissimilarity (n=48 paired samples; p<0.001), we found that correlations exist between species in the nose and (other) species on the skin.

Conclusion

The results show that both the nasal and skin microbiome are associated with disease severity in children with AD. Next to staphylococci, other species contribute to this association.

INTRODUCTION

Changes in the microbial composition of the skin and nose have been suggested to contribute to the complex pathogenesis of atopic dermatitis (AD).[1] The microbiome of the skin in AD is characterized by an increased abundance of *Staphylococcus (S.) aureus*.[2-4] A recent systematic review on the skin microbiome in AD also reports changes in other bacterial species.[5] For example, *S. epidermidis* has been found increased on the lesional skin, and reductions in *Propionibacterium* and *Streptococcus* were reported during AD flares.[3,5,6] These microbial alterations were reported in small single studies and need further validation.[5] Prospective studies observed increased skin colonization with *S. aureus* at the age of 3 months in infants who later develop AD.[7] Another small study also found an association between skin colonization in the antecubital fold with commensal staphylococci (*S. epidermidis and S. cohnii)* at the age of 2 months, and AD later in life.[8,9] Although data are still limited, these prospective studies suggest that the skin microbiome might contribute to both (severity of the) inflammation in AD and the development of the disease.

There is also evidence that the nasal microbiome is involved in the pathogenesis of AD. For example, it has been shown that patients with AD are almost five times more likely to carry *S. aureus* in the nose compared to healthy controls.[2] In a large birth cohort study, colonization of the nares with *S. aureus* at the age of 6 months and frequent colonization during the first year of life were associated with AD and its severity.[10] However, this was not confirmed in other studies.[3,11] Studies into the nasal microbiome in AD are often limited to *S. aureus* and there is very little known about the nasal microbiome in children in relation to AD severity.

The identification of species that contribute to the pathogenesis of AD is important for the development of new specific treatment strategies that aim to modulate the microbiome. Also, knowledge about which microbial niches are involved in AD and how they interact is needed to guide these developments. Several studies described the importance of relations between the nasal and skin microbiome with regard to *S. aureus*.[4,12,13] The anterior nares could be an important reservoir for self-contamination and bacterial spread from the nose to the skin or vice versa. However, the relation between the skin and nasal microbiome in AD has barely been studied before.

In this study we aimed to characterize the nasal and skin microbiome composition in children with AD and determined its association with AD severity. Additionally, we explored differences and correlations between the nasal and skin microbiome.

METHODS

Study design and patients

This cross sectional study was embedded in a randomized controlled trial that compared group consultation with individual consultations in children with AD, treated in the outpatient clinic of the Wilhelmina Children's Hospital of the University Medical Center Utrecht (ISRCTN08506572).[14] Inclusion criteria were: diagnosis of AD according to UK Working Party criteria, age between 0 and 18 years and parental ability to answer Dutch questionnaires.[15] Microbial swabs of the nose and skin, eczema severity scores and patient characteristics were all obtained at baseline and analyzed in the current study. The medical ethical committee of the University Medical Center Utrecht approved the study (08-368/K) and written informed consent was obtained from all participants. Severity of the AD was estimated using the self-administered eczema area and severity index (SA-EASI) by a research nurse.[16] Patients with a SA-EASI from 0 to 17.35 were classified as mild eczema, from 17.35 to 46.27 as moderate eczema and higher than 46.27 as severe eczema, calculated based on cut-offs of the SCORAD score (Supplementary methods).[17]

Microbial samples

A total of 90 microbial samples were taken from the mucosal surfaces of the anterior nares. Skin microbial samples were taken from the lesional skin (n=108), preferably the antecubital fold or popliteal fossa. Samples were collected by a trained research nurse, according to a standardized procedure and using a sterile swab (Sterile Dryswab™) moistened with sterile NaCl 0.9%. After collection, the samples were aliquoted and frozen at -20^{0}C until further processing.

DNA isolation and qPCR

For DNA isolation approximately 150 ul of cutaneous or nasal material (retained by rinsing the swabs in lysis buffer) was directly transferred to the DNA isolation plate. Then 0.5 mL phenol pH8.0 (Phenol solution, catalogue P4557, Sigma-Aldrich, St Louis, MO) was added and the samples were mechanically disrupted by bead beating 2 times 3 minutes with a 96-well plate Beadbeater (Biospec Products, Bartlesville). Samples were centrifuged at 1880 rcf (4000rpm) for 10 minutes to separate the aqueous and phenolic phases. The aqueous phase was transferred to a new 96-well plate and DNA was purified with the AGOWA mag Mini DNA Isolation Kit (AGOWA, LGC genomics, Berlin, Germany) in accordance with the manufacturer's recommendations. After elution, total load of *S. aureus* and *S. epidermidis* was assessed by quantitative (q)PCR using the following primers and probe: 16S-S.aur-F1 (5'-GCG AAG AAC CTT ACC AAA TCT TG-3') and 16S-S.aur-R1 (5'-TGC ACC ACC TGT CAC TTT GTC-3'), 16S-S.aur MGB Taqman® probe (5'-CAT CCT TTG ACA ACT CT-3') with a FAM label, 16S-S.epi-F1(GCG AAG AAC CTT ACC AAA TCT TG) and

16S-S.epi-R1 (CAT GCA CCA CCT GTC ACT CTG T) and the 16S-S.epi MGB Taqman probe (CCT CTG ACC CCT CTA G) with VIC label.

16S rRNA sequencing

The microbial composition of each sample was characterized by mass sequencing of the V4 hypervariable region of the 16S rRNA gene on the Illumina MiSeq sequencer (Illumina, San Diego, CA). To prevent over-amplification, barcoded DNA fragments spanning the Archaeal and Bacterial V4 hypervariable region were amplified with a standardizing level of template DNA (1 ng). These amplicons, generated using adapted primers F515 and R806 (using 30 PCR cycli), were bidirectionally sequenced using the MiSeq system.[18,19] After removing samples with less than 1000 sequences, 89 nasal and 60 skin samples remained (figure S1). Pre-processing and classification of sequences was performed using modules implemented in the Mothur V.1.31.1 software platform.[20] Sequences were trimmed between 243-263nt and chimeric sequences were identified per sample using UCHIME in de novo mode and removed.[21] Sequences with 97% sequence similarity or higher were grouped into operational taxonomic units (OTU) using MOTHUR. Taxonomic names were assigned to all sequences using the Ribosomal Database Project (RDP) naïve Bayesian classifier with confidence threshold of 60% and 1000 iterations and the mothur-formatted version of the RDP training set v.9 (trainset9_032012).[22] For each OTU, the most common sequence was selected as the most representative sequence. Read counts for each OTU were tabulated for downstream analysis. Standardized mock communities were included to check for technical performance of all experimental steps. Negative control samples of the lysis buffer did not show signs of contamination. Based on preliminary cluster analyses we identified three outlier samples in the skin database (data not shown). Two of these samples were dominated by contaminant species (*Bifidobacterium, Enterobacter*) likely transferred via the feces as the samples were collected from the legs of young children. The third skin sample was dominated by *Enhydrobacter*. The three samples were excluded and a total of 89 nasal and 57 skin samples remained for further analysis (figure S1).

Statistical analysis

Differences in baseline characteristics and metadata were statistically tested using the Chi-Square or Fisher's exact test and non-parametric Mann-Whitney U Test for independent samples where appropriate (SPSS version 24). Alpha diversity of the nasal and skin samples was calculated based on unfiltered OTU tables that were subsampled to the sample with the lowest total read count (1160). We calculated richness (number of different OTUs) and Shannon index (number of different OTUs and how evenly they are distributed) and compared these between nose and skin samples using non-parametric independent sample Mann-Whitney-U test. Non-subsampled OTU tables were filtered

for further analysis. OTUs present in less than two samples and with less than 10 counts in total were excluded for downstream analysis. Species and phylum relative abundances were visualized using stacked bar charts. The most dominant species in the nose and skin were estimated based on median relative abundances.

The filtered OTU tables were standardized using Hellinger transformation for further ordination analysis.[23] To test whether AD severity (SA-EASI) significantly drives differences in overall microbial composition, we used a permutational multivariable analysis of variance (PERMANOVA) based on Bray-Curtis dissimilarity.[24] The Bray-Curtis dissimilarity scale measures similarity between communities based on the taxa present and their relative abundances. The PERMANOVA tests were adjusted for age, use of antibiotics and location of sample collection (only skin), and the number of permutations was set on 10000. To identify which species drive the association between the overall microbial composition and AD severity, we obtained PERMANOVA coefficients.[24]

To visualize overall differences in microbial composition between nose and skin samples, we used nonmetric multidimensional scaling (NMDS) plots based on Bray-Curtis dissimilarity. Statistical significance of this difference was assessed using PERMANOVA (10000 permutations). To identify correlations between the microbial communities of the nose and skin, we carried out regularized canonical correlation analysis (RCCA), including the 'ridge' method.[25] For this RCCA we included OTUs that were present in at least 20% of the samples.

The statistical analysis were performed using the R statistical software (RStudio version 1.0.153). We used the packages 'vegan' (version 2.4-6) for NMDS and PERMANOVA [24], 'phyloseq' (version 1.21.0) for alpha diversity [26], 'CCA' (version 1.2) for RCCA [27] and 'ggplot2' (version 2.2.1) for visualization.[28] The set.seed function was used (with seed = 32) to obtain reproducible results.

RESULTS

Characteristics of the study population

A total of 90 nose and 108 skin samples were collected and all 108 skin samples were analysed using qPCR. A total of 89 nose and 57 skin samples were available for analysis after 16S rRNA sequencing (figure S1). The 57 skin samples were collected from the antecubital fold (n=36), popliteal fold (n=9), head/neck (n=5), arm (n=4) and an unknown location (n=3). For 48 children, samples of both the nose and skin were available. Baseline characteristics of the children are described in table 1.

Table 1. Patient characteristics

	Nose cohort (n=89)	Skin cohort (n=57)	p- value
Female sex			
n (%)	42 (47.2)	25 (43.9)	0.694
Age			
months; median (IQR)	30 (11.1-70.6)	22.9 (9.5-62.5)	0.475
SA-EASI			
median (IQR)	30.8 (16.2-52.5) [&]	36.9 (19.2-58.5) ^	0.346
Use of medication, n (%)			
Topical corticosteroids	67 (75.3)	45 (78.9)	0.609
Systemic corticosteroids	0 (0)[$]	0 (0)^	-
Topical antibiotics	7 (7.9)	3 (5.3)	0.741
Systemic antibiotics	2 (2.2)	1 (1.8)	0.663

^ n=55; 2 missing, [&] n=88; 1 missing, [$] n=87; 2 missing

P-values were calculated using Chi-Square test and Fisher's Exact Test for categorical data. A non-parametric Mann-Whitney U Test for independent samples was used for continuous variables

Microbial diversity of nose and skin in children with AD

A total of 2107454 high quality sequences were obtained from the 89 nasal samples (median per sample 22196; range 697-56436). The sequences were assigned to 390 OTUs, 10 different bacterial phyla and one archaeal phylum. Nasal samples contained mainly Proteobacteria (52%), Firmicutes (28%) and Actinobacteria (11%), illustrated in figure 1a. There seems to be a negative correlation between Proteobacteria and Firmicutes, since samples with a high abundance of Firmicutes have lower abundance of Proteobacteria and vice versa. From the 57 skin samples, 1110689 high quality sequences were obtained (median per sample 12449; range 1124-106087), belonging to 358 OTUs of 9 different bacterial phyla and one archaeal phylum. Firmicutes were very predominant on the skin samples with a median relative abundance of 92% of all the bacterial phyla. Proteobacteria, Actinobacteria and Bacteroidetes followed (figure 1b). A comparison of the 10 most abundant OTUs between nose and skin is presented in figure 2. As shown in this figure, *Moraxella, Dolosigranulum* and *Corynebacterium* were prevalent in the nasal microbiome with median relative abundances of 25%, 12% and 7%, respectively. In the skin samples staphylococcal species comprised a large part of the microbial community with a median relative abundance of 87% (figure 2 and figures S2a-b). Alpha diversity, the diversity within a single sample as measured using richness and the Shannon index, shows lower Shannon diversity but not richness in the skin samples compared to the nose samples (figure 3; p-value <0.001 and 0.366).

Figure 1a. Distribution of phyla in the nose samples (n=88, one missing sample)

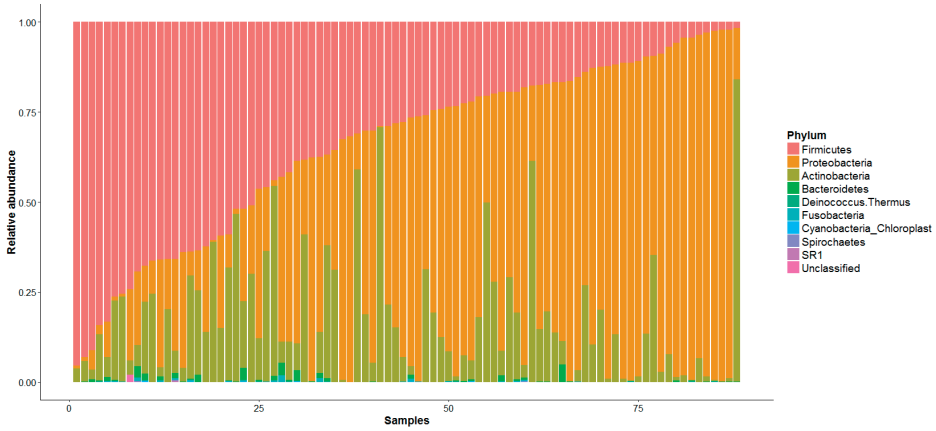
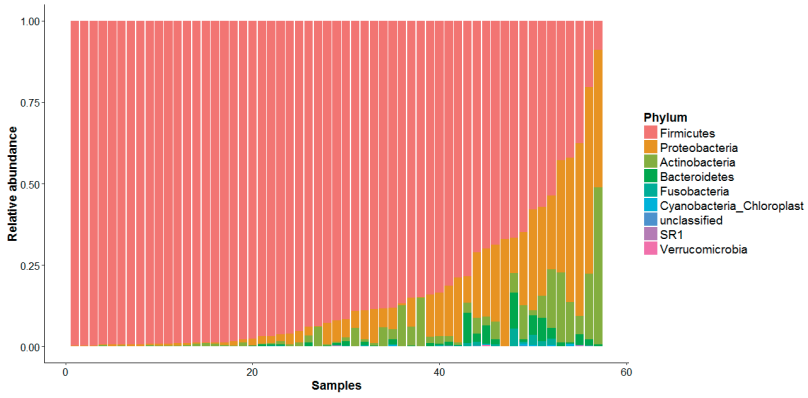


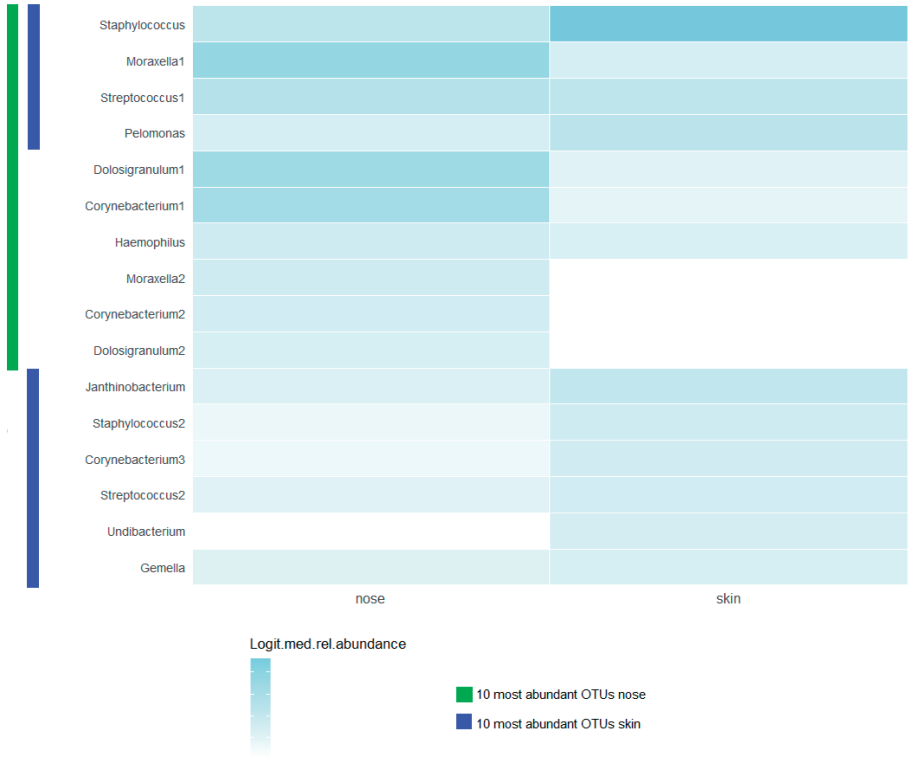
Figure 1b. Distribution of phyla in the skin samples (n=57)



Associations between the microbiome and severity of AD

We found a significant association between the overall microbial composition in the nose and the severity of AD measured by SA-EASI (PERMANOVA, R^2=2.6%; p=0.017; table 2). This association was driven by *Staphylococcus* (increased in more severe AD) and *Dolosigranulum* (decreased in more severe AD) (figure 4a). Also the variance in microbial composition of skin samples was significantly influenced by severity of the AD (R^2=7.0%, p=0.005). Staphylococci were the strongest drivers of the association between skin microbial composition and AD severity. However, other species illustrated in figure 4b might also be implicated. Interestingly, the contribution of the skin microbiome to AD severity is larger than of the nasal microbiome. Next to disease severity, age of the patient was also significantly associated with nasal microbial composition while use of antibiotics and location of sampling influenced the skin microbiome (table 2). To get more insight in the role of the staphylococci on the skin in AD severity, an additional qPCR was performed on the 108 collected skin samples to identify *S. aureus*

Figure 2. 10 most abundant OTU's in the nose and on the skin (n=48)



NOTE: heatmap is based on the logit transformed median relative abundance. Top of blue color gradient bar corresponds with a median relative abundance of 90%. The lowest value (white) lays below 0.01%. On the OTU level, different members of the same species were identified that could not be further classified. The OTUs were named by the species followed by a number (e.g. *Moraxella* 1 and *Moraxella* 2).

and *S. epidermidis* (as a representative for coagulase-negative staphylococci). Of these samples, 50.0% and 79.6% were positive for *S. aureus* and *S. epidermidis,* respectively. Children with moderate and severe AD were colonized with *S. aureus* on their skin more often than children with mild AD (58% and 51% versus 39%), however this was not significantly different (p=0.299; Chi-Square test) and the load of *S. aureus* did not increase with AD severity (p=0.291; figures S3a-b). The prevalence of skin colonization with *S. epidermidis* did not differ between mild, moderate and severe AD (86%, 74% and 80% respectively; p=0.513). However, the load of *S. epidermidis* was significantly higher in severe AD compared to mild AD (p=0.016; figures S3a-b).

Differences and correlations between nasal and skin microbial communities

To visually inspect differences in the microbiome composition between skin and nose we grouped all samples from patients of which both a nose and skin sample were avail-

Figure 3. Richness (Chao1) and Shannon diversity in nose and skin

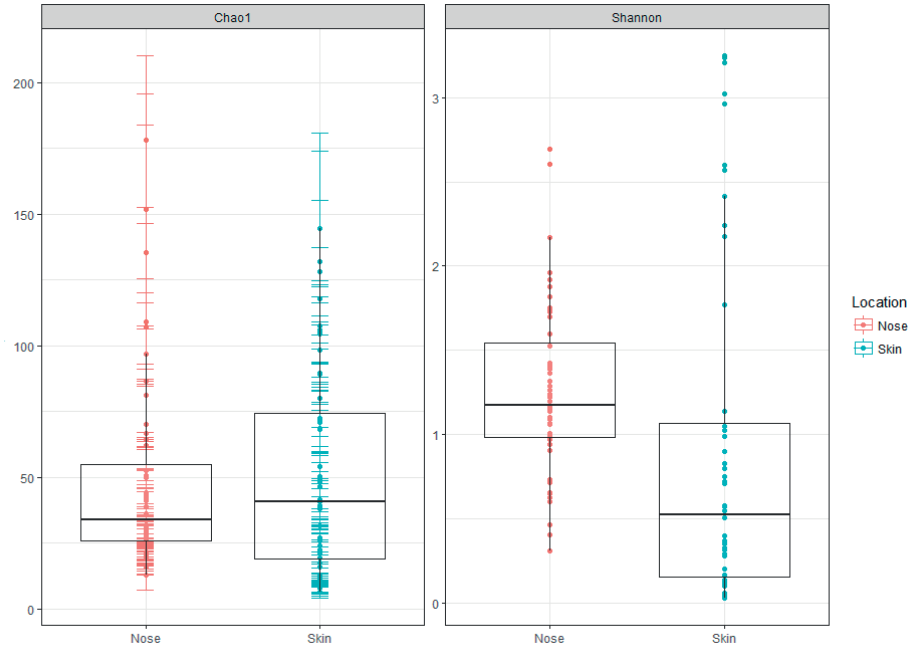


Table 2. Multivariate analysis showing the influence of different covariates on the variance in the nasal and skin microbiome

	Nose (n=88)		Skin (n=53)	
	R^2	P- value	R^2	P- value
SA-EASI #	0.026	0.017*	0.070	0.005*
Age	0.057	<0.001*	0.021	0.212
AB use (topical and oral)	0.006	0.839	0.062	0.007*
Location of sampling ^	-	-	0.052	0.017*

* significant p-value

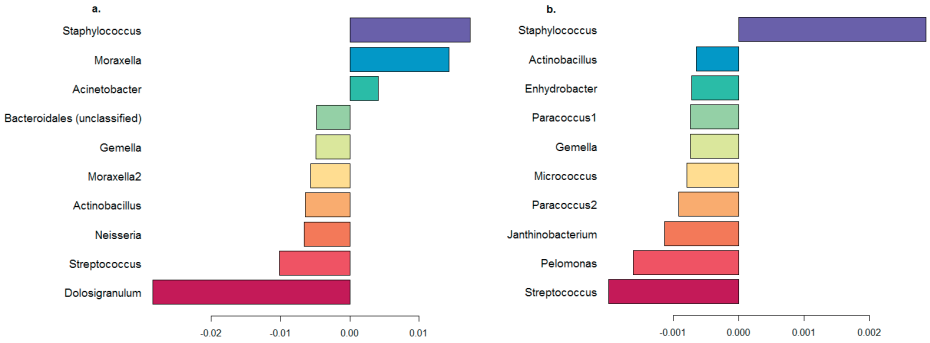
Predetermined order of variables included in the adonis model: SA-EASI, age and antibiotic use for the nose; SA-EASI, sample location, antibiotic use and age for the skin.

^ NB: the homogeneity of dispersion (assumption for adonis) was tested among locations of sampling. P-value of 0.714 indicates that our test results are not an artifact of differences in microbial composition between different locations (beta diversity). As only 4 patients used antibiotics, dispersion could not be reliably tested for these groups.

NOTE: 53 of 57 skin samples were included for analysis due to missing sample location (n=3) and missing SA-EASI score (n=2), one overlapping. 88 nose samples were included, due to one missing SA-EASI score.

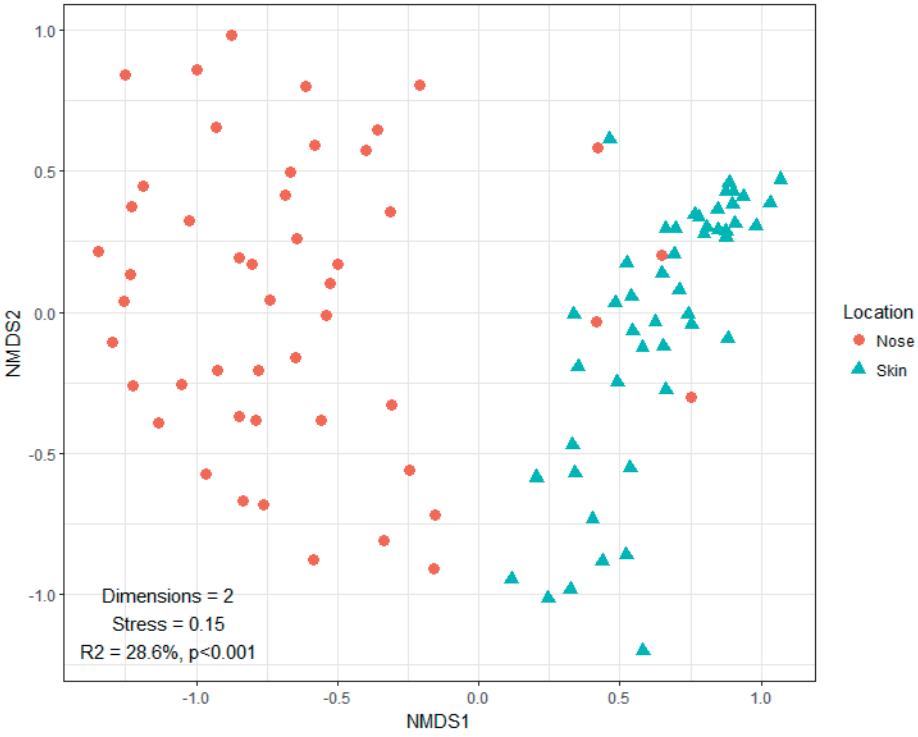
able (n=48). As shown in figure 5 microbial communities of the nose and skin clustered separately (PERMANOVA: R^2=30.9, p<0.001), indicating distinct microbial communities at each location. In all 48 patients, staphylococci were found both in the nose and on the

Figure 4. Top 10 OTUs driving the association between microbial composition and disease severity in nose (a) and skin (b)



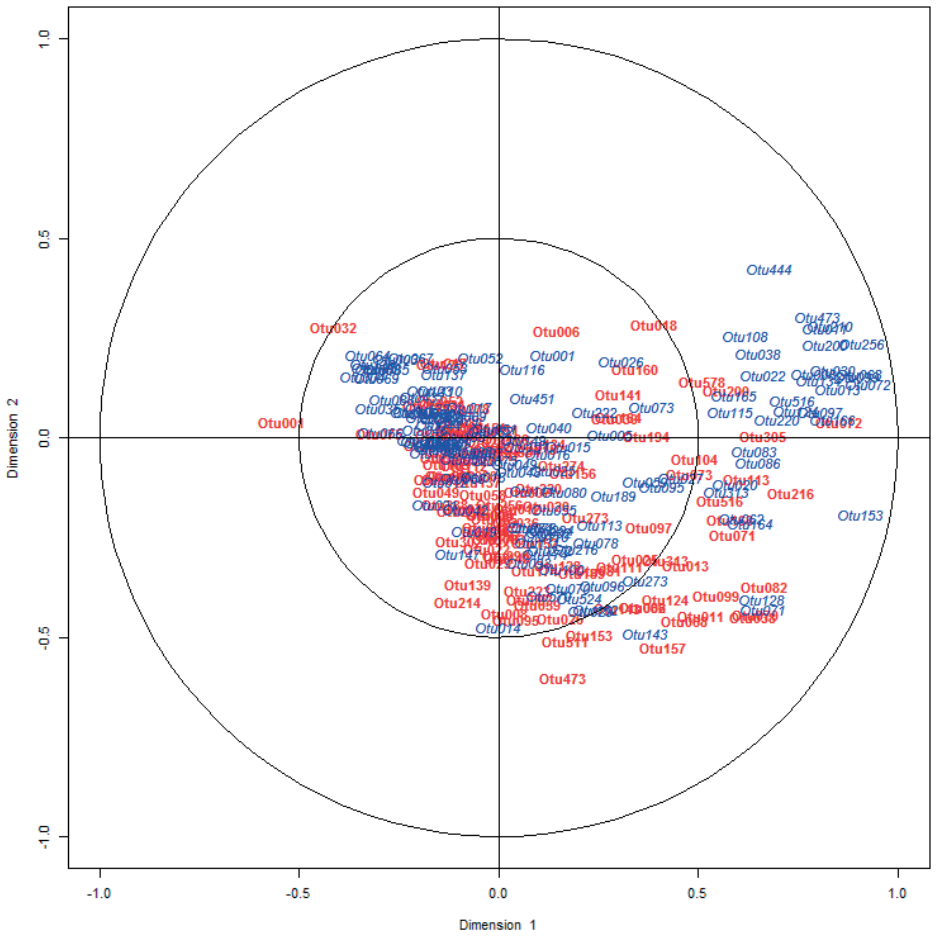
NOTE: The x-axis displays coefficients that represent the contribution of a certain OTU to the association between microbiome composition and severity of the AD.

Figure 5. NMDS plot of skin and nasal samples (n=48). Data are standardized using Hellinger transformation.



NOTE: The microbial composition of a small group of nasal samples resembled the skin samples. A closer view at these four samples showed that these were the samples with highest relative abundance of staphylococci in the nose.

Figure 6. Results from RCCA analysis showing the correlations between the microbiome composition of the nose and skin at OTU level.



NOTE: Canonical correlation dimension 1 and 2: 0.99 and 0.98. Lambda's were 0.00005 and 0.00003. Red OTUs represent the skin and blue represent the nose. Pairs of variables with relatively large weights in the same direction represent positive correlations and variables whose weights have opposite directions exhibit inverse correlations.

skin, but the predominance of the bacterium on the skin was larger (figure S2a-b). Other OTUs that were often found in both niches included *Moraxella* (n=45), *Streptococcus* (n=41), *Pelomonas* (n=39) and *Dolosigranulum* (n=35). To identify correlations between nose and skin microbiome composition, RCCA analysis was performed. Canonical correlations of the first and second dimension were 0.99 and 0.98, indicating that a significant correlation exists between the nasal and the skin databases (figure 6). Most OTUs are placed around the center of the biplot, indicating that these OTUs have a very small

contribution to the correlations between the nose and skin samples (in the first two components). Many skin and nasal OTUs are placed in the same quadrant of the biplot, meaning that they show similar patterns of abundance over the samples. Exceptions are OTU1 and OTU32 in the left upper quadrant, both representing *Staphylococcus* on the skin, that seem to be negatively correlated with most nasal and other skin OTUs.

DISCUSSION

In this study we found distinct microbial communities in the nose and on skin of children with AD. Additionally, we found significant associations between the microbial composition of both the nose and the skin and AD severity in children. Interestingly, this analysis was adjusted for important confounders including age, use of antibiotics and location of sampling.

Our finding that the nose and skin harbor distinct microbial communities is in accordance with earlier studies in healthy subjects.[29] The nares of the children in our cohort were predominated by the phyla Actinobacteria (*Corynebacterium spp.),* Proteobacteria (mainly *Moraxella* but also other species such as *Haemophilus*) and Firmicutes (*Staphylococcus*, *Streptococcus* and *Dolosigranulum* spp.) This is consistent with literature in healthy subjects that describes that the microbiome of the anterior nares is typically enriched for members of these three phyla.[30,31]

Skin lesions were clearly dominated by staphylococci, consistent with literature about skin microbiome in AD.[3,32] While the nasal samples also showed staphylococci, only a few patients showed staphylococcal dominance in the nose. Other OTUs that followed *Staphylococcus* in abundance on the skin of our patients were *Pelomonas*, *Streptococcus* (described especially in young children with AD before) and *Janthinobacterium* (described as an important member in healthy skin microbiome by Grice *et al.*).[32-34] Although the nose and skin harbor distinct microbial communities based on Bray–Curtis dissimilarity, we found that correlations exist between species in the nose and (other) species on the skin. The mechanisms that underlie these findings are not clear yet. Possible mechanisms, such as cross-transmission of bacteria between the niches, need further exploration.

Additional qPCR of the skin samples, showed *S. aureus* and *S. epidermidis* in 50% and 80% of our population, respectively. The prevalence rate of 50% for *S. aureus* on lesional skin is lower compared to a meta-analysis reporting 70%. This might be due to the young age of our cohort (median age of 2.5 years compared to 14 years in the meta-analysis). Kennedy *et al.* also found low *S. aureus* in 2 month old patients with AD.[2] Another study included infants with AD in the first year of life and found positive lesional skin swabs in 21% of the infants, with culture-based analyses.[11] In our cohort, the mean age of the

children that were positive for *S. aureus* did not significantly differ from the group that was negative (Students *T*-test, p=0.705).

We found that both the nasal and the skin microbial composition were associated with AD severity. Cross-sectional studies described associations between an altered microbiome and AD severity before for the skin.[3] For the nose, a single prospective study supports our hypothesis that the nasal microbiome plays a role in (severity of) inflammation in AD. The authors found a relation between nasal colonization with *S. aureus* at 6 months and the development of AD.[10] The timeframe of (nasal) colonization might be important as colonization earlier in life (1 month) was not found correlated with AD development.[11] The strength of the associations between AD severity and microbial composition of the nose and skin was 2.6% and 7.0%, which can be regarded of significant relevance as a large part of the variance in the microbiome can be be explained by host variation.[35] Although an association does not mean causality, our results suggest that both microbial niches (in terms of OTU composition), might play a role in aggravating or worsening the inflammation in pediatric AD. Longitudinal studies that include sampling around AD flares would be needed to confirm if changes in the microbiome precede an increase in AD severity.

Determination of the species that drove the associations between OTU composition and AD severity, showed that in both niches increased staphylococci were of influence. We further classified staphylococci to the species level and found that children with severe AD had *S. aureus* present on the skin more often than patients with mild AD (58% vs 39%) as described earlier.[2] However, this difference was not statistically significant. The presence of *S. epidermidis* on the skin did not significantly differ between mild and severe AD, but the load of *S. epidermidis* was significantly higher in severe AD compared to mild AD. This relates to an earlier study that observed increased bacterial counts of *S. epidermidis* preceding disease flares.[5] Besides staphylococci, we identified other species that drove the association between microbial composition and AD severity. For example, *Moraxella* was found to positively contribute to the association in the nose. An increased abundance of *Moraxella* in the nares of children was not associated with severity of AD before. However, Depner *et al*. found *Moraxella* associated with asthma.[36] In our cohort, 28% of the children had symptoms of asthma or bronchial hyper reactivity (<6 years) reported in their medical file. The association between *Moraxella* and AD severity that we found was not influenced by these diagnosis (p=0.645, data not shown). Thereby, certain species were identified of which a decreased abundance contributed to the association with AD severity in our study. For example *Dolosigranulum* in the nose and *Streptococcus* on the skin (figure 4a-b). High abundance of *Dolosigranulum* was suggested to be beneficial for respiratory health before.[37] *Streptococcus* was observed in lower relative abundance in skin lesions (compared to non-lesional skin) and during flares in children before.[3,32] Future studies should further evaluate the influence of these

species on a lower taxonomic level, as for example different streptococci can vary widely in pathogenic potential.[38] The identification of specific species that contribute to AD and its severity (or protect) is of importance for the development of new treatment strategies for AD.[39,40] Our results can guide future studies in determining their focus for further species specific research.

This study has several limitations. The cross-sectional design of this study precludes statements on cause–effect relationships between microbiota and disease severity. We amplified the V4 variable region using the 806R primer, an often used method at the time of analysis. However, this method does not allow classification of staphylococci at the species level, and recent literature describes that it can be improved by the use of the V1-V3 region or the use of a modified V4 primer.[41, 44] To investigate associations between staphylococci and disease severity to the species level, we performed additional qPCR on *S. aureus* and *S. epidermidis*.[41] Because we were not able to further classify staphylococci into other species, we might have missed correlations between specific staphylococcal species and AD severity.[9] This also holds for strain specific associations with disease severity, as suggested by Byrd et al.[42] In our study *Propionibacterium* was poorly detected in both the skin and nasal samples, while they are described as part of the healthy microbial communities.[43] With regard to the skin samples, the low *Propionibacteria* can still be a true reflection of the eczema lesions as staphylococci are known to overgrow other species. Thereby, we sampled mainly the antecubital and popliteal folds which are known for a low abundance of *Propionibacteria* that prefer more sebaceous environments, such as upper chest.[45] In our study we did not characterize the fungal microbiome, which was found altered in patients with AD before.[5] Despite the limitations, this study shows the relevance of the nasal microbiome in AD and is as far as we know the first to characterize relations between the nose and skin microbiome in AD.

CONCLUSION

This study shows that the severity of AD in children is influenced by the nasal microbial composition. Besides *Staphylococcus*, also other species seem to contribute to the association between skin and nasal microbiome and AD severity. It is important that future studies further explore the role of microbial species in AD, their interaction with the host and other species, and the interaction between different microbial communities of the body, using controlled longitudinal cohorts and adequate sequencing and culture based techniques.

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SUPPLEMENTARY MATERIAL

Figure S1. Flowchart of the sample collection

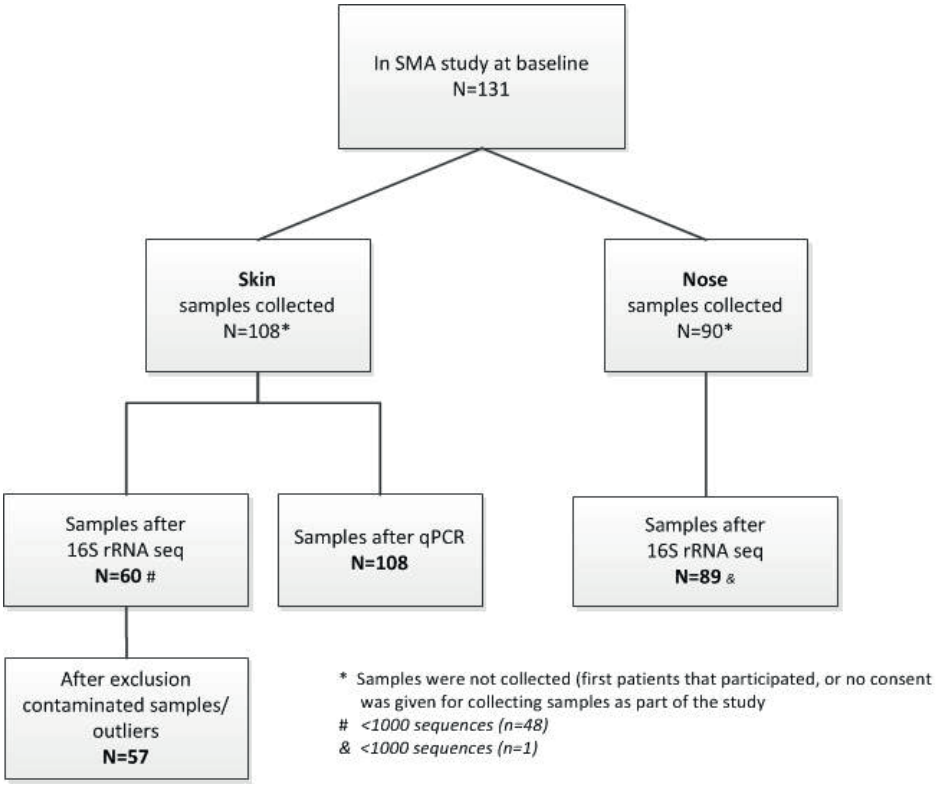


Figure S2a. Distribution of 20 most abundant OTUs in the nose samples (n=89)

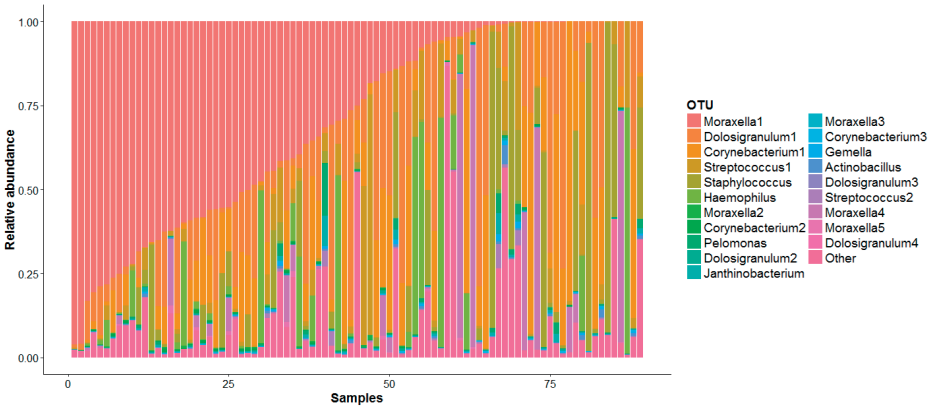


Figure S2b. Distribution of 20 most abundant OTUs in the skin samples (n=57)

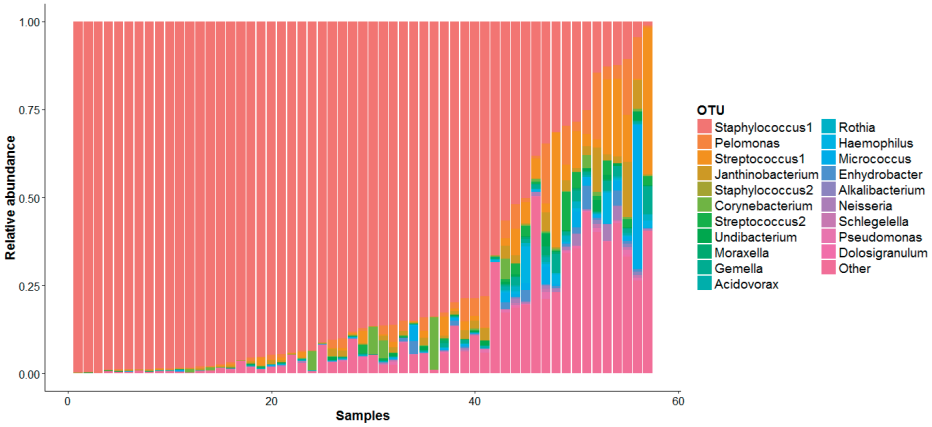
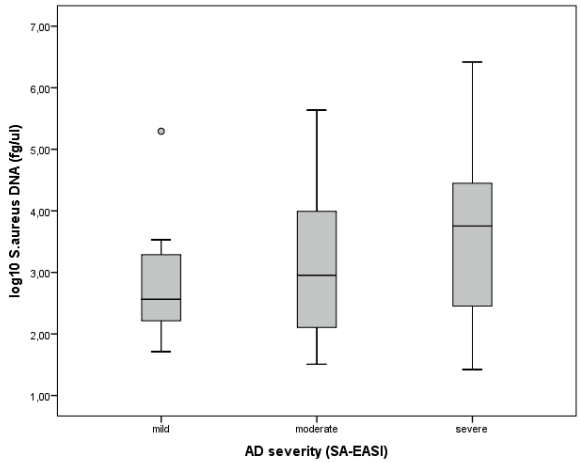
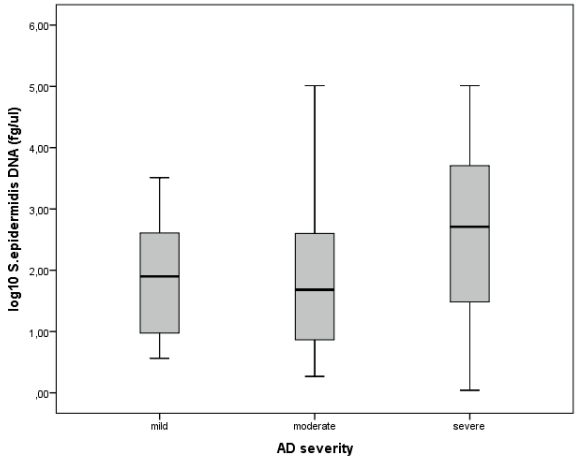


Figure S3a. *S. aureus* load in mild to severe AD



NOTE: no significant differences (p=0.291; ANOVA)

Figure S3b. *S. epidermidis* load in mild to severe AD



NOTE: significant differences (p=0.029; ANOVA), mild versus severe AD (p=0.016; Students t-test)

Supplementary methods

Cut-off values were calculated for the SA-EASI based on the objective SCORing Atopic Dermatitis (SCORAD), a clinical tool used to assess the severity of eczema.[17] The formula to calculate the cut-off points for the SA-EASI included the cut-off points of SCORAD divided by maximum score of SCORAD multiplied by maximum score of the SA-EASI (SA-EASI = cut-off point SCORAD / 83 * 96). Patients with a SA-EASI from 0 to17.35 were classified as mild eczema, from 17.35 to 46.27 as moderate eczema and with a SA-EASI higher than 46.27 as severe eczema.