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ORIGINAL ARTICLE

Atopic Dermatitis, Urticaria and Skin Disease

Skin pH–dependent *Staphylococcus aureus* abundance as predictor for increasing atopic dermatitis severity

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Abstract

Background: Atopic eczema (atopic dermatitis, AD) is characterized by disrupted skin barrier associated with elevated skin pH and skin microbiome dysbiosis, due to high *Staphylococcus aureus* loads, especially during flares. Since *S aureus* shows optimal growth at neutral pH, we investigated the longitudinal interplay between these factors and AD severity in a pilot study.

Method: Emollient (with either basic pH 8.5 or pH 5.5) was applied double-blinded twice daily to 6 AD patients and 6 healthy (HE) controls for 8 weeks. Weekly, skin swabs for microbiome analysis (deep sequencing) were taken, AD severity was assessed, and skin physiology (pH, hydration, transepidermal water loss) was measured.

Results: Physiological, microbiome, and clinical results were not robustly related to the pH of applied emollient. In contrast to longitudinally stable microbiome in HE, *S aureus* frequency significantly increased in AD over 8 weeks. High *S aureus* abundance was associated with skin pH 5.7-6.2. High baseline *S aureus* frequency predicted both increase in *S aureus* and in AD severity (EASI and local SCORAD) after 8 weeks.

Conclusion: Skin pH is tightly regulated by intrinsic factors and limits the abundance of *S aureus*. High baseline *S aureus* abundance in turn predicts an increase in AD severity over the study period. This underlines the importance and potential of sustained intervention regarding the skin pH and urges for larger studies linking skin pH and skin *S aureus* abundance to understand driving factors of disease progression.

KEYWORDS

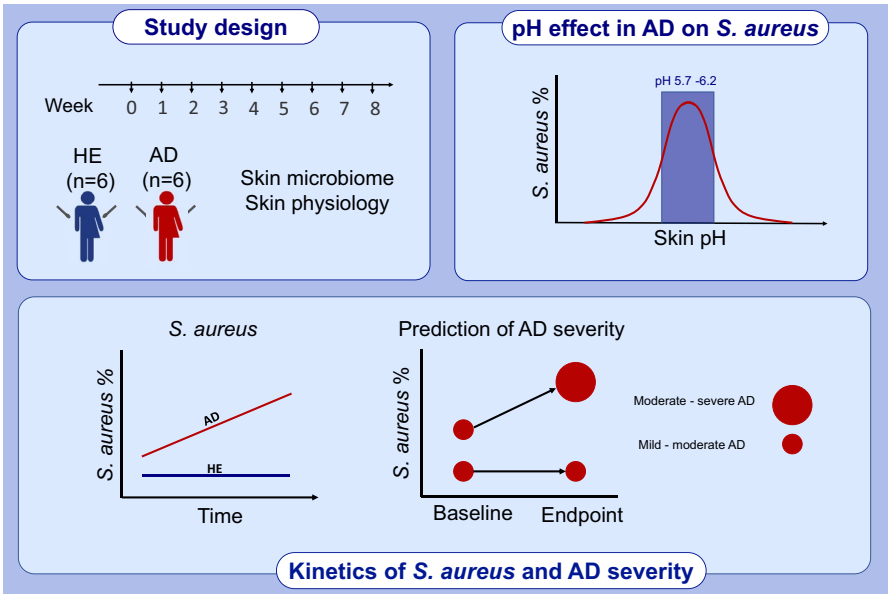
atopic dermatitis, microbiome, pH, skin, *Staphylococcus aureus*

Abbreviations: AD, atopic dermatitis; HE, healthy.

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GRAPHICAL ABSTRACT

In healthy individuals, skin microbiome is stable over 8 weeks, but not in atopic dermatitis patients who show a significant increase in *Staphylococcus aureus* over time. Skin pH of 5.7-6.2 allows the growth of *S aureus* in atopic dermatitis individuals. Higher *S aureus* abundance at baseline is a predictor for increased *S aureus* and AD severity at endpoint.

Abbreviations: AD, atopic dermatitis; HE, healthy.

1 | INTRODUCTION

Atopic eczema (atopic dermatitis, AD) is the most common chronic inflammatory skin disease in children and accepted as risk factor for the development of allergic sensitization.[1,2] Barrier disruption is one central pathophysiological parameter underlying AD. Skin barrier and skin homeostasis are formed by microbiome, chemical, physical, immune, and neurological barriers that form an interactive network.[3] Within this tightly regulated network, skin pH holds a central role. AD is associated with an elevated skin pH, especially during disease flares compared with the naturally more acidic pH in healthy skin.[4] Furthermore, the skin microbiome is dysbalanced and its diversity reduced typically due to an elevated *Staphylococcus aureus* load, which correlates with disease severity.[5–7] However, the interplay between the skin microbiome, physiological skin barrier properties, and AD severity especially in a longitudinally setting is not yet well-understood.

The acidic skin pH is crucial for a functional skin barrier.[3,4,8] Nevertheless, the data of a "physiologic" skin pH vary in the literature most likely due to endogenous and exogenous factors influencing skin pH as well as differences in the measurement procedure. On forearms, foreheads, or cheeks of healthy volunteers, a rather acidic pH (<6) was measured in several studies.[9] The elevated skin pH in AD is linked to disease severity.[10,11] As part of the cutaneous connected network, the skin pH regulates the activity of certain enzymes, such as serine proteases and β-glucocerebrosidases. These enzymes are involved in the regulation of cohesion proteins and in lipid processing in the stratum corneum of the epidermis.[3,8] Lower pH values are associated with less scaling and higher hydration levels, whereas increased skin pH can impair stratum corneum integrity and skin barrier function.[8,9] Thus, the altered skin pH may contribute to the disrupted skin barrier observed in AD patients.

Furthermore, loss-of-function mutations of the barrier protein gene filaggrin (FLG) are known risk factors in the pathogenesis of AD.[12,13] A disruption of the skin barrier is also associated with higher transepidermal water loss (TEWL), which may facilitate the penetration of allergens leading to inflammation and worsening of the disease.[14,15] High TEWL and low hydration are commonly observed in AD skin and often associated with an elevated skin pH.[16] Interestingly, FLG degradation products are skin pH reducing organic acids which act as natural moisturizing factors and reduce *S aureus* growth in vitro.[17–20]

Homeostatic skin microbiome is important to maintain host defense against pathogens and is therefore another important part of the skin barrier.[21–23] While healthy skin demonstrates high microbiome diversity, with *Cutibacterium acnes* and *Staphylococcus epidermidis* being the most common species, in AD skin diversity is significantly reduced due to *S aureus* dominance.[21–23] Furthermore, the skin pH is part of the microenvironment regulating bacterial growth. At acidic (<5.5) pH growth of *S epidermidis* is enhanced, whereas the growth of *S aureus* and Propionibacteria is suppressed.[9,24] *S aureus* in turn grows best at neutral (6-7) pH conditions.[25]

Considering both healthy and AD-related dysbalanced microenvironments, we focused on the possibility of influencing skin pH through emollient application with different pH. Following our hypothesis that an emollient with pH 5.5 supports physiological skin barrier functions and inhibits the growth of harmful *S aureus*, we performed a longitudinal intervention pilot study with a comprehensive approach involving not only clinical but also skin physiological (pH, hydration, TEWL) and microbiome analysis. Additionally, we hypothesized that skin pH and *S aureus* abundance effect changes in AD

disease severity over time. This is to the best of our knowledge the first study of this nature.

2 | METHODS

2.1 | Study design

The study was designed as a double-blinded intra-individual comparison of two emollients that only differ in their pH values: either acidic (pH 5.5) or alkaline emollient (pH 8.5). Each emollient was applied on one randomized body side (left or right) within a certain test body region. Six healthy individuals (HE) and 6 AD patients were enrolled for the pilot study. Participants applied the emollients twice daily in the morning and evening and returned to the study center on weekly basis for clinical evaluation (Figure S1). The study was approved by the ethics committee of the Technical University of Munich (187/17S). All participants signed informed consent statements. Examinations took place from October to December 2017 (four HE: IDs 3-6 and four AD: IDs 11-14) and from April to June 2018 (two HE: IDs 1 and 2 and two AD: IDs 15 and 16).

2.2 | Inclusion/exclusion criteria

A hypersensitive reaction against the study emollients was excluded by epicutaneous patch tests and assessment after 48 hours. Reddening or papulation of the skin led to exclusion. Exclusion criteria were antibiotic treatment <14 days before the start of the study or application of steroids or calcineurin inhibitors within the region of interest or systemic use <7 days prior to the start of the study. Therefore, only AD patients with mild-to-moderate SCORAD < 40 (min = 12.8, max = 37.6, average = 29.4) with comparable severity of eczema on both body sides in at least one body area, where study emollients were later applied later. Body test regions of HE were matched according to AD and were distributed as follows: 2× dorsal lower leg, 2× antecubital areas, 1× volar upper arm, and 1× volar shoulder. Study groups were matched in age and gender.

2.3 | Emollients and application procedure

All study emollients were provided by Sebapharma GmbH & Co. KG. Participants were asked not to use any other skin care products or topical treatment beside the study emollients in the determined body region. For the application of each emollient, a new glove was used each time. Emollients were applied widely in the test region in a field of about 10 × 15 cm. On average, 4.7 mg of emollient, comparable in both study groups and treatment arms, was applied per cm^2. Time distance from measurements and samplings to the last emollient application was at least 3 hours and to the last shower at least 12 hours. Emollient pH was measured at the beginning and end of study period.

2.4 | Physiological measurements

Measurements were taken after an acclimatization period for the skin of 20 minutes at a mean room temperature of 22.2°C and a mean humidity of 46.6 g/m^3. Transepidermal water loss (TEWL), hydration, and skin pH were measured with Tewameter® TM 300, Corneometer® CM 825, and Skin-pH-Meter PH905 by Courage + Khazaka Electronic GmbH. The mean out of 3 measurements was taken for statistical analysis, respectively. General severity of AD was evaluated using objective SCORAD,[26,27] EASI,[28] or local symptoms within the test region (details Methods S1).

2.5 | Microbiome analysis

For microbiome analysis, skin swabs (Sigma-swab, MWE) were taken dry, rubbing 20 times in a field of 2 cm × 2 cm, and stored in 500 µL Stool DNA Stabilizer Solution (Stratec) at −80°C and analyzed using 16S (variable region V1-V3, primers 27F-YM and 534R) amplicon-based next-generation sequencing. For DNA extraction, the QIAamp UCP Pathogen Kit (Qiagen) was used. Lysis was performed using Precellys Evolution (Bertin Technologies®: Montigny-le-Bretonneux, France). The 16S rRNA sequencing was performed on Illumina MiSeq® platform (Illumina Inc) using 2 × 300 bp pair-end reads (MiSeq® Reagent Kit v3 600 cycles; Illumina Inc). OTU clustering against the SILVA database was performed using CLC Genomics Workbench 11.0.1 and its microbial genomics module. After quality control (merging, chimera reduction), samples with less than 1000 reads were excluded from analysis (2 samples from HE_ID_6, week 1, 6 - pH 5.5 or pH 8.5, respectively). The α- and β-diversity and frequency of the top 10 families and species were analyzed based on the RHEA R-scripts published by Lagkouvardos et al 2017.[29] Optimized species annotation was performed using the algorithm of Bhattacharyya et al 2020.[30] For detailed protocol, see Methods S1.

2.6 | Statistical analysis

Statistical significance of differences in continuous variable results between the HE and AD groups was assessed by the nonparametric Mann-Whitney test, while differences between the 2 emollient applications (left and right arms of the same patients) were assessed by the paired Wilcoxon nonparametric test. Since changes in skin microbiome were independent of the emollient pH, data from the 2 body sides were combined for analyses of differences between AD and HE groups and longitudinal analysis. However, the results are qualitatively the same also when analyzed separately for each of the emollient applications. For longitudinal analysis, the mean of all measured physiological, clinical, and microbiome parameters of early (weeks 0-2), mid (weeks 3-5), and late (weeks 6-8) phases of the study was calculated for samples of each body side separately to reduce fluctuations in the data. Correlation between continuous variables was assessed using the nonparametric Spearman rank

test. *P*-values were considered as significant at the alpha-error two-tailed level of $P < .05$ and robustly significant with $P < .03$ over a few measurements. Unless otherwise stated, median values (with maximum and minimum) are shown in the figures. Statistical analysis and plots were created either with R or GraphPad Prism version 6.00 for Windows, GraphPad Software.

3 | RESULTS

3.1 | Patient demographics

Participant groups were age- and gender-matched. Each group consisted of 2 male and 4 female participants with a mean age of 43.5 in HE and 43.35 in AD. All participants were Caucasian (Table S1).

3.2 | Skin pH buffered by intrinsic regulatory mechanisms

At baseline, both body sides exhibited similar skin pH (Figure 1A), TEWL (Figure 1B), and skin hydration (Figure 1C) in each of the study groups, AD and HE. During the intervention only at a few time points, slightly lower skin pH was measured in the pH 5.5–treated body sides compared with the pH 8.5–treated sides in both study groups. The difference in skin pH between treatment groups was statistically significant after weeks 2, 5, and 7 in AD and only after week 6 in HE ($P < .05$). However, the skin pH did not reach emollient pH levels. No effect of the pH level of the emollient on TEWL (Figure 1B) or hydration (Figure 1C) was observed neither in HE nor in AD.

3.3 | Skin physiology differences between AD and HE

There was no significant difference in skin pH (Figure 1A) between HE and AD in this study, neither at baseline nor during the time course of the study. TEWL (Figure 1B) was significantly higher in AD (median over study period: 27.4) at all time points compared with HE (9.3) (Table S2). Skin hydration was generally lower in AD (AD 27.8, HE 46.6); however, at baseline the difference was not significant (Figure 1C).

Independent of the pH of the emollient, none of our study groups showed major changes in parameters of skin physiology compared with baseline values (week 0).

3.4 | Individual changes in disease severity of AD

At baseline, as well as at later time points, no difference in local SCORAD (Figure 1D) was seen between the pH 5.5- and pH 8.5–treated body sides. Furthermore, compared to baseline, local SCORAD did not significantly change in the time course of the study. Whereas overall per group the scores for EASI and objective SCORAD remained stable from baseline to endpoint, some participants increased remarkably in objective SCORAD (5 of 6) and EASI (4 of 6) (Figure 1E,F).

3.5 | No differences in skin microbiome between application of emollient with pH 5.5 or 8.5

At baseline, no differences were detectable between the later differently treated body sides in none of the study groups, neither in β-diversity (Figure S2) nor in α-diversity (Figure 2A,B). Analyzing the taxonomic composition at baseline, the top 10 families and species were generally comparable on both body sides in AD and HE (Figure 2D). Furthermore, no robust differences between the body sides due to emollient pH were seen in the microbiome α-diversity indices richness and evenness as well as on family (Figure 2A-D) and species levels (Table S3) at any time point. At endpoint, the global microbiome remained unaltered irrespective of the emollient pH (Figure S2).

3.6 | Differences in the skin microbiome between HE and AD

Comparing the global microbiome of HE and AD at baseline, different clusters were visible, which remained throughout the study (Figure S2). No difference in α-diversity indices was seen at baseline. The main difference between AD and HE over time was higher *S aureus* abundance on species level and lower Micrococcaceae on family level in AD (Figure 2E, Table S3).

3.7 | Stable skin microbiome in HE contrasts increase in *S aureus* abundance in AD

Whereas richness and evenness were stable upon emollient application in HE participants, the richness in AD patients increased significantly at all time points except for week 3 ($P < .05$) compared with baseline. Over time, the family of Lactobacillaceae significantly decreased in AD patients (Table S3). Whereas *S aureus* abundance increased significantly in all weeks except for week 3 compared with baseline in group AD, HE participants had no change in *S aureus* levels (Figure 2E). When looking at patient level, it becomes clear that only some AD patients have an increase in *S aureus* abundance (Figure 2F).

3.8 | Skin physiological parameters correlate among each other

Skin pH was positively correlated with TEWL ($P < .05$; $r = .63$ (early), $r = .63$ (mid) $r = .65$ (late)). Between skin pH and skin hydration, no significant correlation was detected; however, a negative association was visible ($r = -.4$ (early), $r = -.45$ (mid), $r = -.5$ (late)). Furthermore, TEWL and skin hydration were negatively correlated at early- and mid-time points (early: $P < .05$; $r = -.63$: mid: $P < .05$; $r = -.69$, late: $P = .058$; $r = -.56$) (Figure S3).

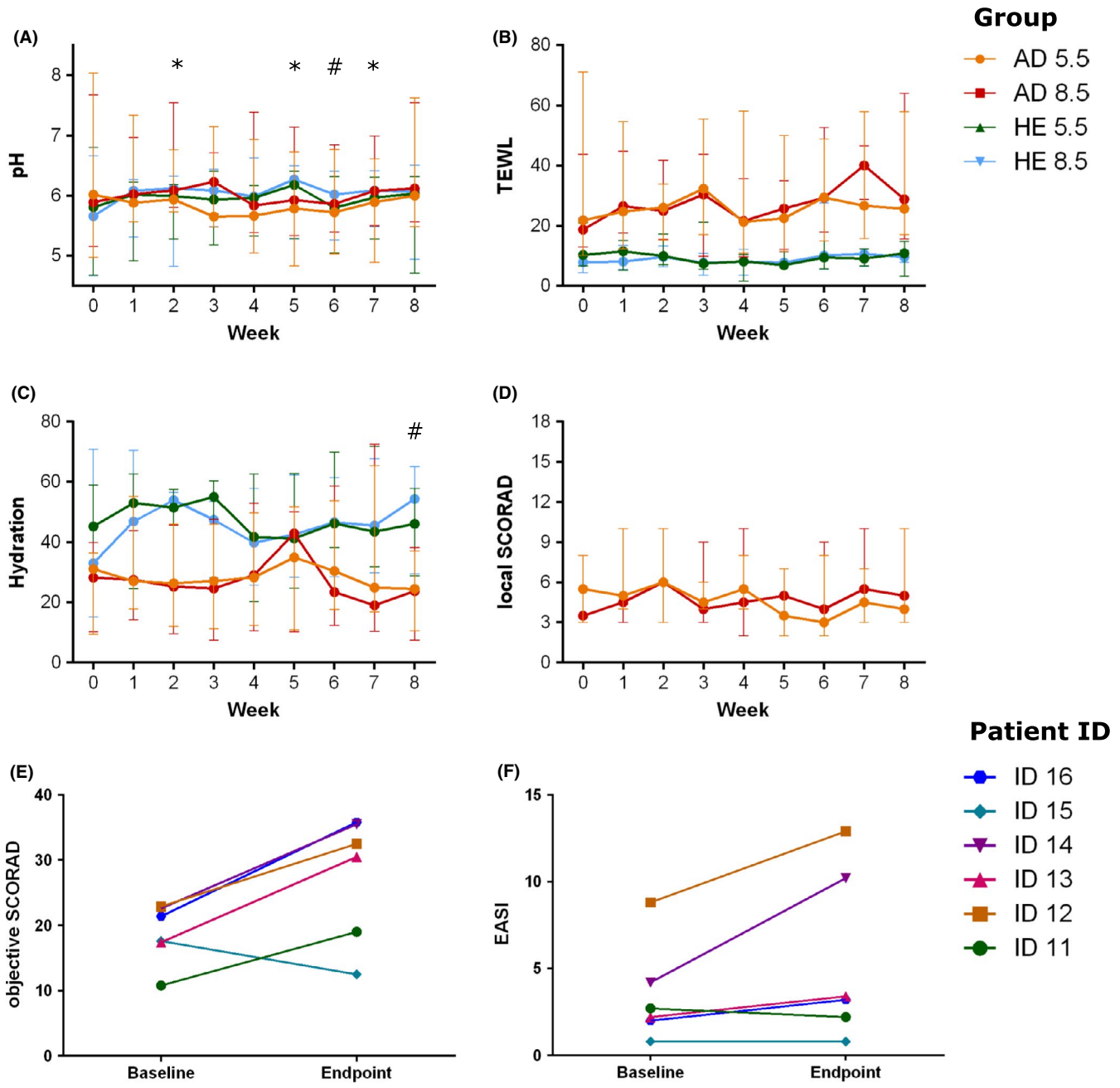


FIGURE 1 Skin physiology and clinical scores. Emollient pH does not robustly affect skin physiology. Changes in physiological parameters (skin pH (A), transepidermal water loss (B), and skin hydration (C)) over the study period of 8 wk are shown as median (min, max) per group and treatment (AD 5.5 = orange, AD 8.5 = red, HE 5.5 = green, HE 8.5 = blue). In AD patients, changes in local AD severity in treated body sides (local SCORAD) are shown over 8 wk (D). Additionally, the general AD severity indexes objective SCORAD (E) and EASI (F) are shown at baseline (week 0) and at endpoint (week 8) of the observation period per AD patient. Significant difference between treatment (emollient pH 5.5 and 8.5) is shown as * (AD) or # (HE). *P*-values for comparison of treatments, groups, and to baseline are shown in the Table S2

3.9 | pH and hydration influences *S aureus* growth in AD patients

Staphylococcus aureus abundance higher than 10% was only found in the pH range of 5.7-6.2 (Figure 3 A-C), while lower or higher skin pH only allowed lower *S aureus* frequency. Increasing *S aureus* from early to mid- to late phases also occurred only in the same pH range, in association with higher local SCORAD. Whereas TEWL seemed to have no relation to *S aureus* abundance (Figure 3D-F), low hydration was associated with higher *S aureus* abundance in the early phase ($r = -.66$, $P < .05$), with the same trend observed in mid- and late phases (Figure 3G-I).

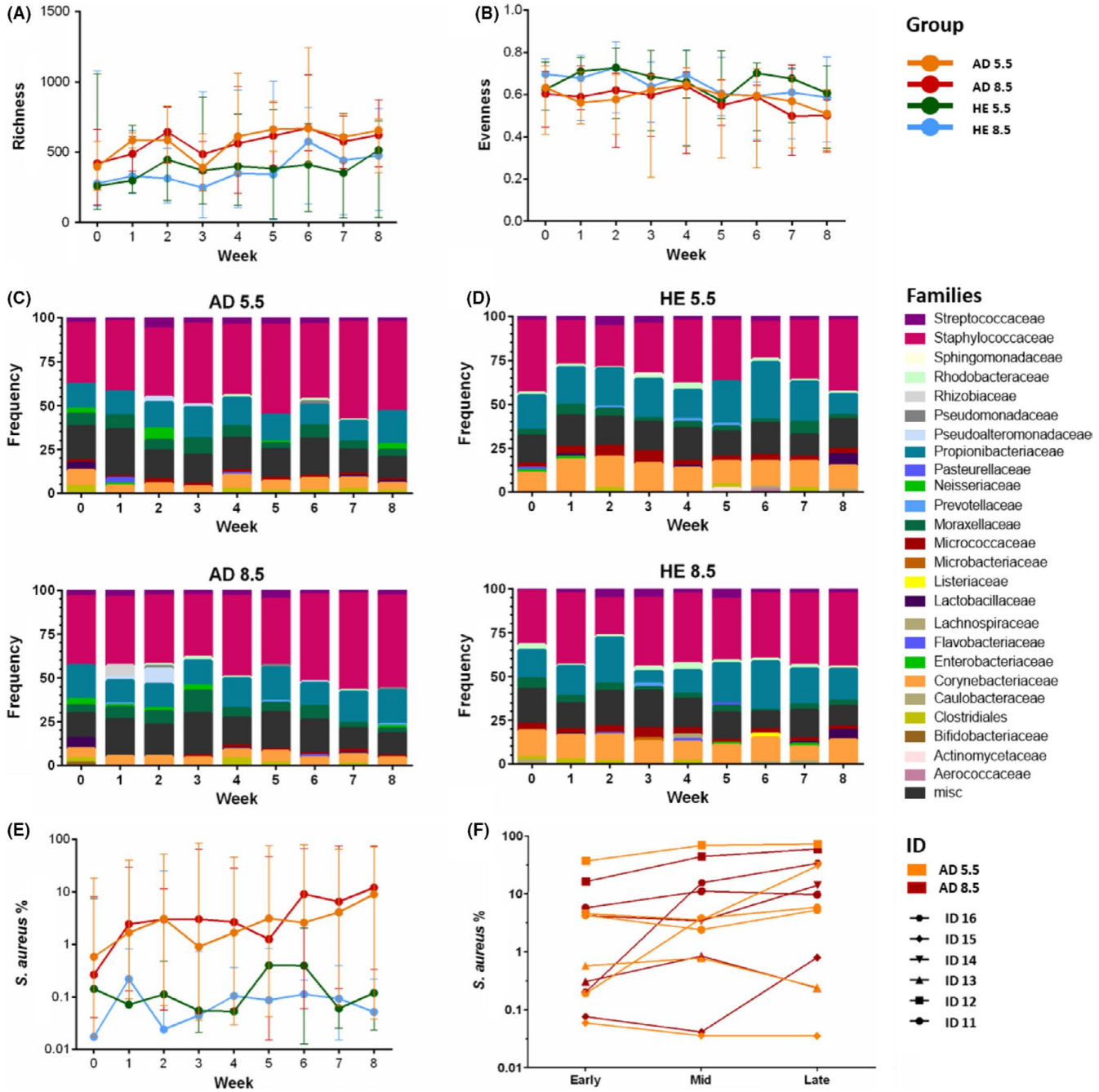


FIGURE 2 Skin microbiome. Skin microbiome is stable in HE, while in AD *Staphylococcus aureus* abundance significantly increases, independently of emollient pH. The α-diversity indices richness (A) and evenness (B) as well as *S aureus* abundance (E) are shown over the study period of 8 wk as median (min, max) per group and treatment (AD 5.5 = orange, AD 8.5 = red, HE 5.5 = green, HE 8.5 = blue). Top 10 taxa at family level are shown for AD (C) and HE (D) separately for the treatments. Individual *S aureus* abundances of all AD participants are shown at early (weeks 0-2), mid (weeks 3-5), and late (weeks 6-8) phases of the study (F). *P*-values for comparison of treatments, groups, and to baseline are shown in the Table S3

3.10 | High *S aureus* correlates with local severity of symptoms

High *S aureus* abundance was strongly correlated ($r = .79$, $P < .01$) with higher individual local SCORAD in mid and late phases of the study (Figure 4B,C), but not in early phase (Figure 4A). Generally, *S aureus* was lower in early phase and its increase in mid- and late phases was associated with an increase in local SCORAD (Figure 5A,B).

3.11 | Baseline *S aureus* abundance as predictive factor for increasing disease severity

Intriguingly, high *S aureus* abundance at baseline was correlated with an increase in *S aureus* from baseline to endpoint ($r = .78$, $P < .01$). Furthermore, increase in severity of EASI and local SCORAD could be predicted by baseline *S aureus* abundance. High baseline *S aureus* resulted in more severe worsening of AD

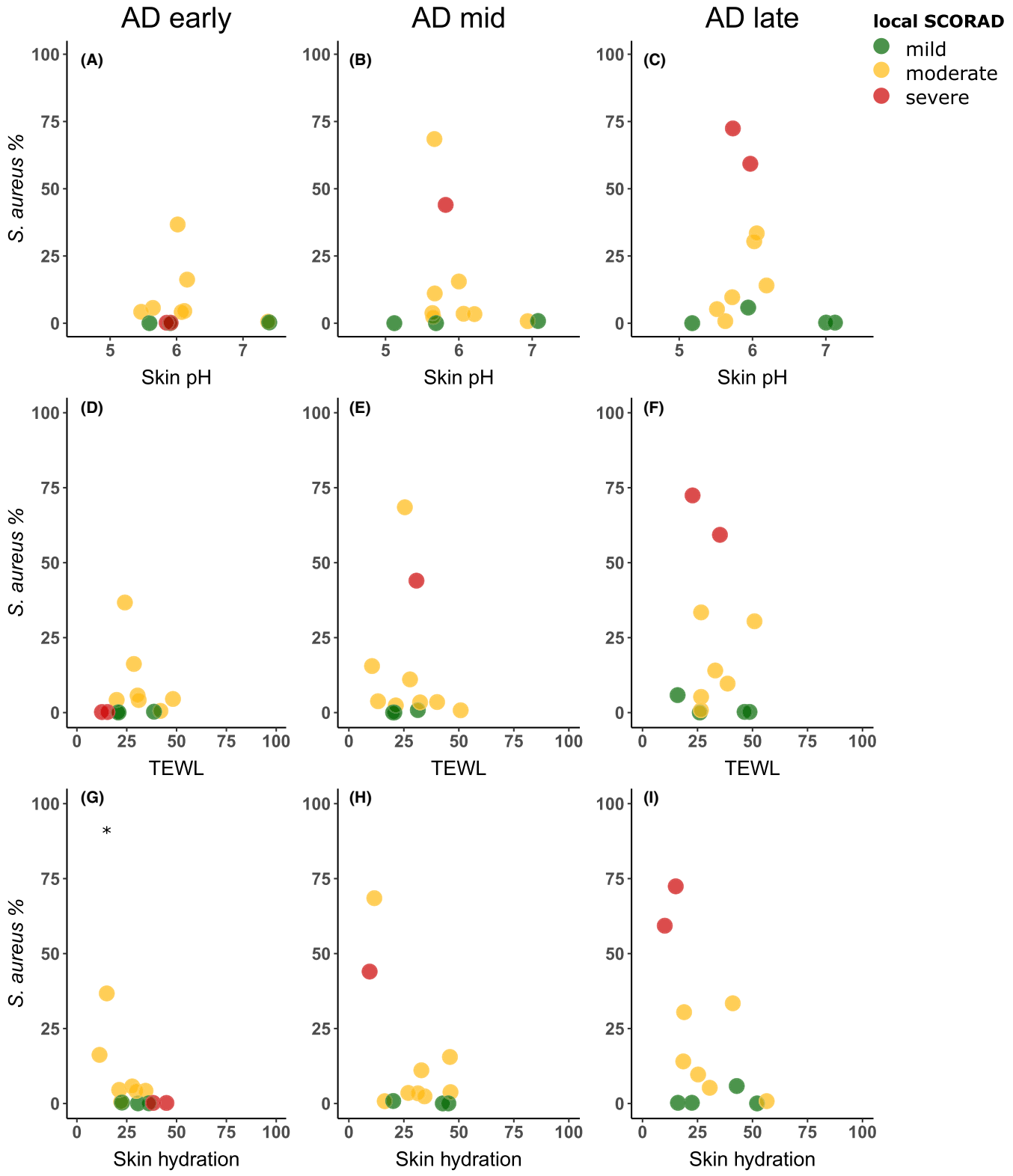


FIGURE 3 Correlations of skin physiological parameters with *Staphylococcus aureus* abundance in AD patients. Skin pH of 5.7-6.2 and low hydration are associated with high *S aureus* abundance. *S aureus* abundance is shown as function of skin pH (A-C), TEWL (D-F), and skin hydration (G-I) at early, mid, and late time points. Colors correspond to local SCORAD values (green = mild (<5), yellow = moderate (5-8), red = severe (>8)). Significant correlations between the physiological parameters and *S aureus* are marked with $^{*}P < .05$

(change local SCORAD: $P < .05$; $r = .59$, change EASI: $P < .05$; $r = .64$). However, objective SCORAD changes were not correlated with baseline *S aureus*. In accordance, high baseline abundance of *S aureus* was correlated with high local SCORAD ($r = .83$, $P < .001$) and EASI ($r = .86$, $P < .001$), but not with objective SCORAD after 8 weeks (Figure S4).

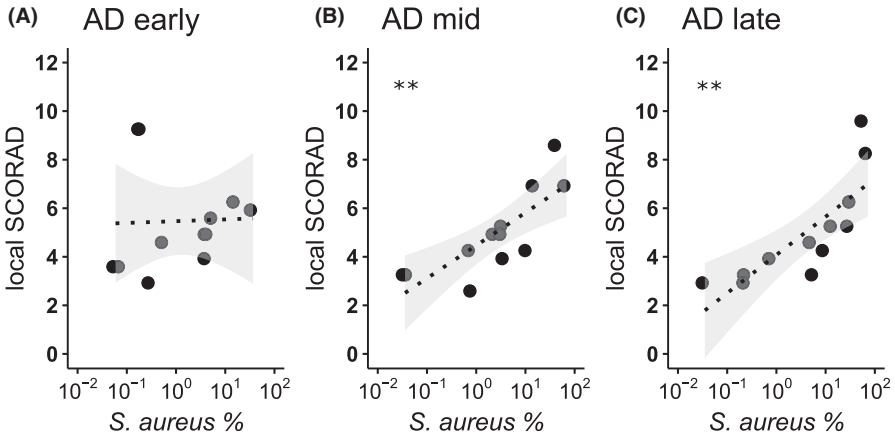


FIGURE 4 Clinical symptoms in the context of *Staphylococcus aureus* abundances in AD. Disease severity is correlated with *S aureus* abundance in mid and late phases of study. Local SCORAD of each individual and body side (black dots) is shown as function of *S aureus* abundance at early (A), mid (B), and late (C) time points. Significant correlations are marked with $^{**}P < .01$

4 | DISCUSSION

This is the first study where frequent longitudinal measurement of skin microbiome was assessed in connection with skin physiology measurements and with AD disease severity during an intervention with a skin emollient. Our data strongly suggest that the skin microbiome of healthy individuals is tightly regulated over time, in contrast to an instable microbiome in AD patients Strikingly, we found that the initial abundance of *S aureus* is predictive for future increase in *S aureus* abundances and in AD severity. Thus, individuals with high abundances of *S aureus* might be more likely to experience naturally occurring flares as previously hypothesized by us.[31] However, this conclusion is only based on a worsening of EASI and was not confirmed by objective SCORAD. However, it should be noted that upon study initiation patients stopped their usual treatment and started the application of a new emollient. These changes in daily skin care habit may be considered as a possible reason for the worsening of AD, but anyway correlated with *S aureus* at baseline.

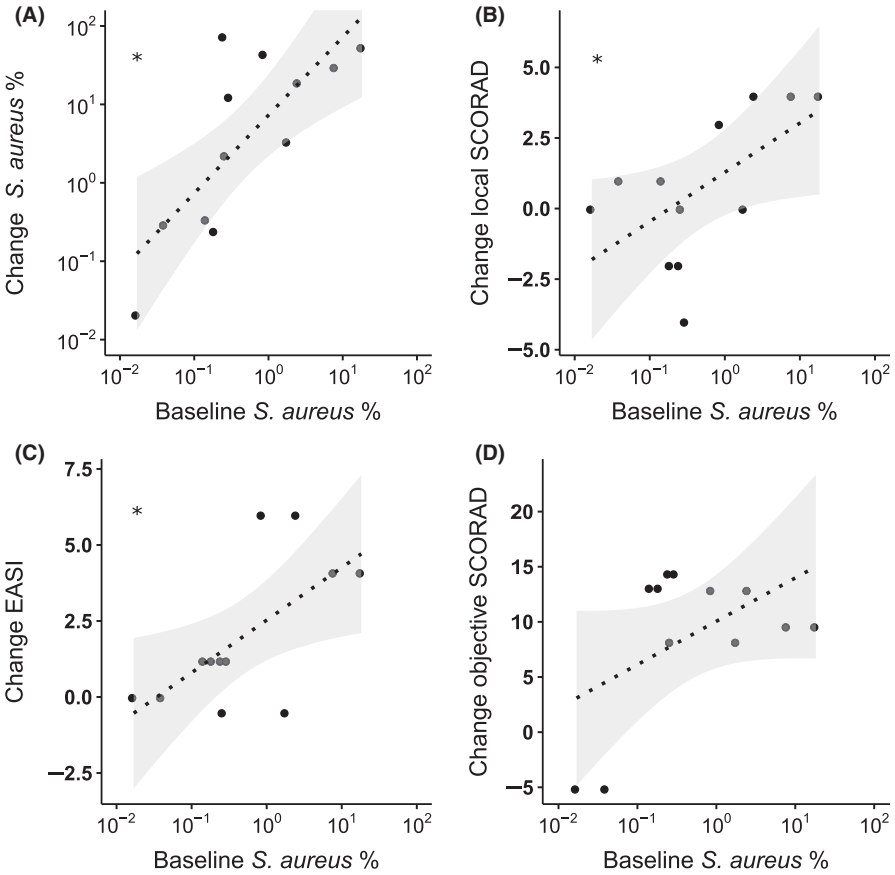


FIGURE 5 Changes in microbiome and AD severity as function of baseline *Staphylococcus aureus* abundance. High baseline *S aureus* predicts worsening of AD severity. Changes (baseline to endpoint) in *S aureus* abundance (A), local SCORAD (B), EASI (C), and objective SCORAD (D) as function of baseline *S aureus* abundance in individual AD patients. Significant correlations are marked with $^{*}P < .05$

Interestingly, local AD severity worsening was not associated with the emollient pH.

With our results, we highlight once more the link between *S aureus* and AD severity.[10,11,32] Some *S aureus* strains are potentially pathogenic and capable of expressing superantigens and enterotoxins,[33,34] which lead to an inflammation cascade additionally damaging the skin barrier,[32] and linking *S aureus* abundance and clinical symptoms in AD.[5,35,36] Increasing bacterial load or clonal expansion of pathogenic strains can play a role. In accordance, high *S aureus* abundances were associated with high severity of local clinical symptoms at mid and end of the study. However, at the early phase of the study, *S aureus* abundance was generally low and not correlated with local clinical symptoms, which might be due to the inclusion of only low-to-moderate AD patients in the study. Also, local SCORAD is highly subjective score and clinical symptoms can be related to other factors such as stress.[37,38]

There is much controversy about the effect of cosmetic, emollients, and basis therapy in general on the skin microbiome and its diversity. Contrasting the stable microbiome in HE, richness increased in AD patients over the study period of 8 weeks using the emollient as described previously.[39,40] Abundance of Micrococcaceae was significantly lower in AD patients in our study confirming previous results.[41] Interestingly, the family Lactobacillaceae, which harbors probiotic species also considered for AD treatment, significantly decreased over the study period in AD patients.[42,43] Lastly, *S aureus* was significantly more abundant in AD patients than HE as previously described.[10,11,32]

We found the highest counts of *S aureus* in a pH range between pH 5.7 and pH 6.2, whereas higher or lower pH value seems to limit its growth. According to the literature, the optimum pH for the growth of *S aureus* is between pH 6 and pH 7, which unfortunately cannot be tested in our study since neutral pH was only represented by one participant with comparable low *S aureus* abundance. Furthermore, it is likely that other factors than pH control *S aureus* growth, as in the same pH range also low *S aureus* counts were found along with very high ones. The microenvironment of the skin also includes the factors dryness and osmolarity which undergo rapid changes and can influence bacterial growth. Low hydration was associated with high *S aureus* growth in our AD patients, whereas osmolarity is an interesting factor which we did not consider in our study.

As expected from the literature, TEWL was higher and hydration level was lower in AD patients and often associated with an elevated skin pH.[14–16] Surprisingly, in our study AD and HE did not significantly differ in skin pH which could be due to relatively high skin pH in HE in our study compared with previous studies and low number of participants.[9,11] Furthermore, skin pH is known to be linked to disease severity and in our study only participants with mild-to-moderate eczema were included.[10,11]

As previously described, skin pH cannot be easily altered sustainably by the study emollients although a short-term change was observed (Figure S5).[9,44] However, especially in AD, a temporary difference in skin pH was observed, hinting toward a reduced buffer capacity in AD. This is of great importance considering that many common skin care products have a rather basic pH.[4] Compared to water baths, dilute bleach baths do not consistently reduce AD severity and *S aureus* load in vivo and are not antimicrobial *in vitro*.[45,46] In the "Guidelines of care for the management of AD" from 2015, rather acidic skin care is advised, more recent publications confirm the benefit of acidification of the skin for improved barrier function in murine models.[47–52] Even a preventive potential was suggested.[49,51] Derived from the results of our study and other previous findings,[49,51,52] products with even lower pH than 5.5 might be needed to change skin surface pH effectively. Alternatively, a more continuous application [51] by increasing the frequency of application could furthermore contribute to reach more sustainable changes in skin physiology.

Even though this is a pilot study, we achieve statistical power due to the study design with intra-individual controls in a double-blinded fashion with frequent longitudinal sampling. However, our findings must be confirmed in a larger validation study.

Slightly acidic emollients are already a fixed tool in the therapy of AD.[4,53] However, according to our results and as expected, basic skin care cannot control disease severity and underlines that basic skin care cannot be the only but a central tool in disease management. If further studies with a larger number of patients confirm the predictive assumptions of *S aureus* abundances in relation to clinical symptoms, it could become a helpful tool for planning therapeutic options in AD.

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CONFLICT OF INTEREST

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AUTHOR CONTRIBUTION

CTH, MR, and GH conceived and supervised the study. The clinical part was performed by KT and the lab work was done by AdT. CH, KT, MB and TN analyzed the data supervised by AUN, MR and GH. CH and KT wrote the manuscript, with input from all authors. CH and KT are equally contributing first authors. AUN, MR and CTH are equally contributing senior authors and CTH corresponding author.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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