

#### **4. Temporal shifts in the skin microbiome associated with disease flares and treatment in children with atopic dermatitis.**

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Atopic dermatitis (AD) has long been associated with *Staphylococcus aureus* skin colonization or infection and is typically managed with regimens that include antimicrobial therapies. However, the role of microbial communities in the pathogenesis of AD is incompletely characterized. To assess the relationship between skin microbiota and disease progression, 16S ribosomal RNA bacterial gene sequencing was performed on DNA obtained directly from serial skin sampling of children with AD. The composition of bacterial communities was analyzed during AD disease states to identify characteristics associated with AD flares and improvement post-treatment. We found that microbial community structures at sites of disease predilection were dramatically different in AD patients compared with controls. Microbial diversity during AD flares was dependent on the presence or absence of recent AD treatments, with even intermittent treatment linked to greater bacterial diversity than no recent treatment. Treatment-associated changes in skin bacterial diversity suggest that AD treatments diversify skin bacteria preceding improvements in disease activity. In AD, the proportion of *Staphylococcus* sequences, particularly *S. aureus*, was greater during disease flares than at baseline or post-treatment, and correlated with worsened disease severity. Representation of the skin commensal *S. epidermidis* also significantly increased during flares. Increases in *Streptococcus*, *Propionibacterium*, and *Corynebacterium* species were observed following therapy. These findings reveal linkages between microbial communities and inflammatory diseases such as AD, and demonstrate that as compared with culture-based studies, higher resolution examination of microbiota associated with human disease provides novel insights into global shifts of bacteria relevant to disease progression and treatment.

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