Research letter

Identification of a unique *Staphylococcus aureus* ribosomal signature in severe atopic dermatitis

DOI: 10.1111/bjd.16936

Dear Editor, Increased colonization by *Staphylococcus aureus* has been demonstrated in approximately 90% of patients with atopic dermatitis (AD). Its prevalence on the skin increases with disease severity when examined using both culture-dependent[1] and culture-independent methods.[2] Microbiome studies, targeting the gene encoding bacterial 16S ribosomal RNA (rRNA), have shown that microbial diversity is inversely correlated with disease stage in both adults[3] and children, and also with the overall proportion of *Staphylococcus* species in children.[4] This trend is reversed with treatment.[4]

While classical microbiome techniques are immensely important in uncovering the connections between bacterial communities and disease states, they rely on clustering 16S sequences that are 97% similar, thereby inherently losing a level of resolution that may be important in resolving strain significance in the disease. Recently we developed a technique that overcomes this shortcoming and enables high-resolution profiling of the 16S gene.[5] The technique, Short MUltiple Regions Framework (SMURF), combines sequencing results from different polymerase chain reaction (PCR)-amplified regions, and therefore provides coherent profiling of a larger DNA fragment (the 16S gene in our case). Full details of the experimental methods are available from the authors on request.

Skin colonization by *S. aureus* can activate the host immune system through multiple cascades, ultimately leading to inflammation. Phenotype singularities have been observed from AD-associated *S. aureus* including elevated δ-toxin expression, biofilm-like colonization of the skin, and its dominance on a suppressed antimicrobial peptide landscape. These observations suggest that AD-associated *S. aureus* is unlike other *S. aureus* strains, including type strains and those isolated from other *S. aureus* pathologies. Given the unique properties of AD-associated *S. aureus* and its ubiquitous dominance on AD skin, we reasoned that there may be genomic signatures inherent to these inimitable *S. aureus* strains. A recent whole-genome *S. aureus* sequencing study posited that a small progenitor population of *S. aureus* clonal complex 1 expands concurrently with AD flares.[6] We sought to determine whether the 16S sequences of AD-associated *S. aureus* can be differentiated between healthy, severe AD and moderate AD skin on both lesional and nonlesional skin.

We recruited a cohort of 35 patients with AD and 10 healthy individuals at the DMZ Medical Center in Ein Bokek, Israel, between March and November of 2015. Details of all participants are available on request. The study was approved by the Helsinki Committee of the Rabin Medical Center, Petah Tikva, Israel, and written informed consent was obtained from patients or their parents or guardians. Patients were sampled prior to the commencement of any therapeutic regimen and avoided washing and emollient treatment in the 12 h preceding sampling. Exclusion criteria for both patients and healthy volunteers included pregnancy and the use of systemic or topical antibiotics over the past month. A complete medical history and skin examination was carried out by a physician, and AD severity was assessed using the Scoring Atopic Dermatitis index. Importantly, our cohort consisted of both children and adults with AD. Samples were taken from the antecubital fossa and popliteal fossa on lesional and contralateral nonlesional sites, and DNA extraction and 16S amplicon libraries were prepared, sequenced and processed as previously described.[7]

Using SMURF,[5] we identified four unique full-length 16S rRNA *S. aureus* sequences, or ribotypes, in our cohort (Fig. 1a). We refer to these as *S. aureus* ribotypes 1, 2, 3a and 3b, where the latter two are indistinguishable along the amplified regions. *Staphylococcus aureus* ribotype 3 (i.e. 3a and 3b) was the only differentially abundant staphylococcal ribotype between moderate and severe AD skin on both the antecubital fossa and popliteal fossa. This ribotype was significantly more abundant in individuals with severe AD on both lesional and nonlesional skin (Fig. 1b–d). The relative abundance of *S. aureus* ribotype 3 was proportional to disease severity and inversely proportional to Shannon diversity (data available on request), on both the antecubital and popliteal fossae, unlike *S. aureus* ribotypes 1 and 2.

This observation complements the plethora of studies that have identified phenotype singularities of AD-associated *S. aureus*, and suggests an underlying genomic variance of AD-associated *S. aureus*. Of additional importance, the only sequence with 100% sequence homology to *S. aureus* ribotype 3 in the entire GenBank, EMBL, DDBJ and PDB databases hails from AD skin.[4] Notably, of the myriad AD skin microbiome studies published in the last 6 years, ours and that of Kong *et al.*[4] were the only ones to examine near full-length 16S sequences, and therefore this particular nuance of the 16S gene could not be resolved in the aforementioned studies.

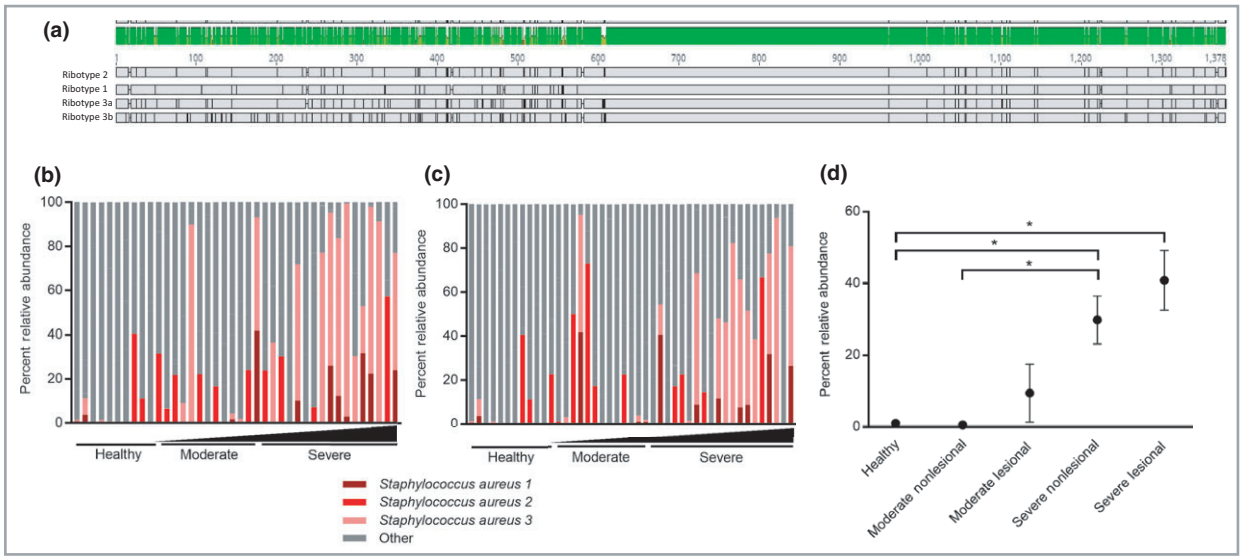

Fig 1. *Staphylococcus aureus* ribotype 3 dominates the microbiome on skin severely affected by atopic dermatitis (AD). (a) 16S sequence dissimilarity between the three dominant *S. aureus* ribotypes. Breaks in the uppermost green line represent variable nucleotides across all sequences. Vertical black lines in each ribotype sequence represent the location on the gene that diverges from the other sequences. (b, c) Taxonomic relative abundance of the three *S. aureus* ribotypes present on (b) lesional and (c) nonlesional antecubital fossa sorted by Scoring Atopic Dermatitis values. (d) Comparison of the relative abundance of *S. aureus* ribotypes between healthy individuals and patients with moderate AD and severe AD on lesional and nonlesional skin. *$P < 0.05$.

These two studies report uncultured sequences, and a cultured *S. aureus* with this 16S signature remains elusive. Undoubtedly, isolating, culturing and sequencing this *S. aureus* can prove instrumental in our understanding of the unique pathogenic capabilities of this *S. aureus* strain. Regardless, identification of a specific strain variant is undoubtedly an important step forward in understanding AD disease aetiology. Our work suggests that *S. aureus* strains found on healthy skin are not the same microorganisms found on AD skin, and that understanding the disease phenotype necessitates an understanding of the physiological capabilities of *S. aureus* ribotype 3.

This finding expands on previous assertions of the emergence of dormant strains of *S. aureus* on AD skin by providing a simpler and quicker method to uncover its presence on the skin. Ribotype-specific primer sets can be used to detect the presence, absence and/or relative abundance of *S. aureus* ribotypes on AD skin via PCR or quantitative PCR. These results advance the concept that *S. aureus*-dominated AD dysbiosis on lesional and unaffected skin is an important prognostic and therapeutic target and opens a pathway towards rapid identification of AD-associated pathogenic *S. aureus*.

Acknowledgments

We would like to thank the volunteers who participated in this study. Additionally, we acknowledge Shira Wax and Ira Mezin for assisting in ethical approval and planning. Thank you to Dr Idit Shiff and Dr Abed Nasereddin of the Hebrew University of Jerusalem Genomic Applications Laboratory, Core Research Facility, Faculty of Medicine, for providing sequencing support.

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Funding sources: this study was supported by the Israeli Ministry of Science and Technology (grant 3-11174). M.B. is a recipient of the Kaete Klausner Fellowship. The funding organizations were not involved in the study design, data collection, data analysis or any other stage of the research.

Full details of the experimental methods are available from the authors on request.

Conflicts of interest: none to declare.

