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Standardizing Hidradenitis Suppurativa Skin Microbiome Research: The Methods Matter

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Abstract

Cutaneous dysbiosis is implicated in hidradenitis suppurativa (HS) pathogenesis. Previous studies reveal skin microbiota shifts in HS lesional skin. In their report, Riverain-Gillet et al. (2020) extended these findings and reported skin microbiota shifts in unaffected HS skinfolds as well. Their study suggests that skin microbial shifts may precede clinical lesions and draws attention to study methods important for skin microbiome research.

Hidradenitis suppurativa (HS) is a disabling chronic inflammatory disease with significant comorbidities and no uniformly effective treatments, affecting an estimated 1% of the Western population (Ingram et al., 2018). Despite its prevalence and significant morbidity, HS biology is poorly understood, and this has limited the development of novel treatments. The characteristic lesions of HS, including painful inflammatory nodules, abscesses, and tunnels with malodorous drainage, have implicated bacteria in the pathogenesis of HS, and antibiotics have been associated with improvement in disease status. Immune dysregulation has also been observed in HS, leading to the US Food and Drug Administration approval of the TNF antagonist, adalimumab, for this disease. Taken together, these observations suggest that chronic dysregulated immune responses to cutaneous dysbiosis may result in persistent tissue inflammation in HS.

In recent years, advances in cutting-edge genomic technologies have illuminated uncultivable microbes in HS. Studies examining the HS skin microbiome have focused primarily on microbial alterations observed at HS lesional sites. Although these studies concur in their reports of decreased relative abundances of skin commensal bacteria and increased relative abundances of mixed anaerobic bacteria at HS lesional sites as compared with controls, they differ in their assessment of differences in bacterial diversity and the taxa

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CONFLICT OF INTEREST

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primarily driving diversity in HS lesions, HS non-lesional skin, and healthy controls (Guet-Revillet et al., 2017;Naik et al., 2020; Ring et al., 2017;Schneider et al., 2020). Two studies have further reported a positive association between HS severity and anaerobic bacteria; however, limited overlap in the abundant organisms driving diversity has been reported (Guet-Revillet et al., 2017;Naik et al., 2020).

In the Journal of Investigative Dermatology (2020), Riverain-Gillet et al. (2020) report the results of a cross-sectional study profiling skin bacteria from clinically unaffected skin of the axilla, gluteal cleft, and inguinal fold from 60 patients with HS and 17 healthy controls using surface swabs for bacterial culture and genomic sequencing. Before specimen collection, study participants abstained from immunomodulatory treatments and topical and systemic antibiotics for one month, topical antiseptics for one week, and deodorants on the day of sampling, but they did not alter their hygiene habits. HS severity was determined by Hurley staging at affected sites. Bacterial taxonomic assignment was performed by amplification and sequencing of the V1-V2 hypervariable regions of the bacterial 16S ribosomal RNA gene.

To date, this work represents the largest study examining microbial shifts in clinically unaffected skin in patients with HS. Extending findings from previous studies examining skin microbiota shifts in HS lesional skin, Riverain-Gillet et al. (2020) observed skin microbiota shifts in clinically unaffected skin as well. These findings suggest that skin microbiota shifts may precede clinical lesions and may be associated with previously observed changes, including hair follicle dilation, follicular hyperkeratosis, leukocytic infiltration, and inflammatory cytokine expression, in clinically unaffected skin of patients with HS (Boer and Weltevreden, 1996;van der Zee et al., 2012;Vossen et al., 2019).

Using conventional culture methods, Riverain-Gillet et al. (2020) observed decreased abundances of skin commensals in the skinfolds of patients with HS, including coagulase-negative *Staphylococcus* and *Cutibacterium acnes* and increased abundances of anaerobic bacteria in the inguinal folds and gluteal clefts of patients with HS. *Corynebacterium spp* abundances correlated with more severe disease, whereas *Cutibacterium spp* abundances inversely correlated with severe disease.

Next, using sequence-based methods, similar to previous studies, Riverain-Gillet et al. (2020) observed that bacterial evenness was greater in HS unaffected sites than in controls (Naik et al., 2020). They found decreased relative abundances of staphylococci and increased relative abundances of anaerobic bacteria in unaffected HS skinfolds, and they determined that these bacteria were the primary drivers of ecological distances between HS and healthy control skin-folds. HS, body mass index, and the sampled site independently associated with increased relative abundances of anaerobic bacteria at unaffected sites. Using hierarchical clustering analysis, Riverain-Gillet et al. (2020) determined that over 60% of HS samples were associated with anaerobic bacteria, including *Prevotella, Porphyromonas*, and *Peptoniphilus asaccharolyticus*. These anaerobic bacteria have individually been associated with microbial shifts at HS lesional sites in earlier studies (Guet-Revillet et al., 2017;Naik et al., 2020;Ring et al., 2017; Schneider et al., 2020). While *Prevotella* and other anaerobic pathobionts previously identified in HS lesions were

significantly more abundant in unaffected HS skinfolds, only *Staphylococcus aureus* was associated with HS clinical severity.

Although this report of cutaneous dysbiosis in clinically unaffected skin of patients with HS is compelling and potentially clinically relevant, it also draws attention to the study methods that are important for skin microbiome research. A standardized approach for conducting skin microbiome research has previously been proposed to ensure that observed microbiota perturbations can be attributed to the exposure under investigation, rather than to unanticipated confounders (Kong et al., 2017). These standards also help to ensure reproducibility of findings across studies. The elements of this approach include recommendations for skin preparation, exposure and clinical metadata collection, and sample acquisition and processing.

Previously described minimum skin preparatory standards limit confounders because of topical and systemic medications, including antibiotics, personal hygiene practices, cosmetic use, and environmental factors, such as deodorant and emollient use and cleansing practices that have previously been shown to perturb the skin microbiome (Kong et al., 2017). Although strict skin preparatory regimens can limit recruitment of study participants, one approach is to provide a standardized hygiene regimen across participants, thereby controlling for hygiene practices within study design and maximizing patient comfort and recruitment. The absence of previously outlined minimum skin preparatory standards or a uniform hygiene regimen in the study by Riverain-Gillet et al. (2020) makes it challenging to determine the variables responsible for the observed skin microbiota shifts in HS unaffected skin.

Even with implementation of skin preparatory regimens, eliminating or standardizing all confounders can be a challenge in human subjects research. This is especially true for skin microbiome studies because of the various environmental agents individuals may be exposed to on a regular basis. Therefore, in addition to standardized skin preparation, it is imperative to collect meticulous clinical and exposure metadata on disease severity, sampled site characteristics, comorbid conditions, personal hygiene practices, lifestyle factors, and environmental exposures to facilitate downstream analyses and interpretation of skin microbiome data (Kong et al., 2017). Appropriately, Riverain-Gillet et al. (2020) points out that omission of metadata on personal hygiene practices, environmental exposures, and previous antibiotic exposures in their study considerably limits the interpretation of their findings.

Collecting negative controls consisting of collection or storage buffers at the time of sample acquisition is critical to confirm the integrity of specimen collection and processing as low bacterial biomass in skin samples puts them at high risk for contamination and amplification artifacts (Kong et al., 2017). The absence of negative controls in the study by Riverain-Gillet et al. (2020) makes it difficult to eliminate variables at specimen collection or processing that may have contributed to the observed skin microbiota shifts.

Finally, selecting appropriate 16S ribosomal RNA hypervariable region targets for amplification and sequencing is critical for accurate detection of bacteria at a given body

site. While the V1-V2 regions are especially important for distinguishing major skin species, including *Staphylococcus* and *Streptococcus spp*, V3 may be important for detection of *C. acnes*, a clinically relevant skin commensal bacterium (Chakravorty et al., 2007). For these reasons, the most commonly sequenced hypervariable regions for skin microbiome studies have been V1-V3 (Kong et al., 2017). As a result, limiting amplification and sequencing to V1-V2 in the study by Riverain-Gillet et al. (2020) may have implications for accurate assignment of bacterial taxonomy for skin microbes.

Although Riverain-Gillet et al. (2020) provides novel and potentially important insights into HS bacterial shifts through their study findings, it will be important to determine whether future controlled studies describe similar shifts in bacterial community composition at clinically unaffected sites in patients with HS. Future longitudinal studies should examine how skin microbiota perturbations shift over the course of waxing and waning disease, if site-specific microbial shifts are maintained longitudinally, and if these shifts portend disease development at body sites. Identification of skin microbiota clusters (or even specific bacterial species) that may serve as biomarkers for disease prognosis or predictors of treatment response could inform clinical practice. Finally, a deeper understanding of the functional impact of specific abundant or unique microbes in HS will be important to advance microbiota-based therapies.

Standardized study design is necessary to ensure the accuracy, reliability, and reproducibility of skin microbiome data across studies. It is also of paramount importance to improve understanding of pathogenesis and to guide successful translation of these findings into clinically relevant biomarkers and therapeutic targets to improve disease management. In addition, methodological standards are imperative to advance HS microbiome studies from single-center efforts to future large-scale, multicenter studies and facilitate incorporation of mechanistic skin microbiome studies into interventional trials. Ultimately, the potential clinical relevance of skin microbiome data depends heavily on implementation of standards that ensure high-quality, reproducible results.

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