

# Longitudinal Study of the Psoriasis-Associated Skin Microbiome during Therapy with Ustekinumab in a Randomized Phase 3b Clinical Trial

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Plaque psoriasis, a chronic inflammatory disease primarily affecting the skin, is thought to have a multifactorial etiology, including innate immune system dysregulation, environmental triggers, and genetic susceptibility. We sought to further understand the role of skin microbiota in psoriasis pathogenesis, as well as their response to therapy. We systematically analyzed dynamic microbiota colonizing psoriasis lesions and adjacent nonlesional skin in 114 patients prior to and during ustekinumab treatment in a phase 3b clinical trial. By sequencing the bacterial 16S ribosomal RNA gene from skin swab samples obtained at six anatomical sites, we identified minor, site-specific differences in microbial diversity and composition between pretreatment lesional and nonlesional skin. During therapy, microbial communities within lesional and nonlesional skin diverged, and body-site dispersion increased, reflecting microbial skin site-specificity. Microbiota demonstrated greater pretreatment heterogeneity in psoriatic lesions than in nonlesional skin, and variance increased as treatment progressed. Microbiota colonizing recurrent lesions did not overlap with pretreatment lesional microbiota, suggesting colonization patterns varied between initial and recurrent psoriatic lesions. While plaque psoriasis does not appear to be associated with specific microbes and/or microbial diversity, this large dataset provides insight into microbial variation associated with (i) disease in different body locations, (ii) initial versus recurrent lesions, and (iii) anti-IL12/23 therapy.

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## INTRODUCTION

Chronic plaque psoriasis is an immune-mediated disease of the skin and joints. Current evidence suggests a multifactorial etiology, including immune system dysregulation, genetic susceptibility, and environmental factors. A growing

appreciation of the role of the innate immune system in psoriasis pathogenesis stems from the prominent role of inflammatory cytokines and cells associated with innate immunity in the disease and associations observed between psoriasis and genetic variations involved in innate immunity (Sweeney et al., 2011).

Because the innate immune system is the first line of defense against invading pathogens and is important for host–microbiota homeostatic relationships (Thaiss et al., 2016), the question of whether a microbial component contributes to psoriasis pathogenesis arises (Fry et al., 2013). Group A  $\beta$ -hemolytic streptococcal throat infections have been associated with psoriasis exacerbations, particularly guttate psoriasis (Leung et al., 1995; McFadden et al., 2009; Telfer et al., 1992); however, anti-streptococcal treatment does not reliably modify disease (Owen et al., 2000). The specific association with streptococcal species led to the hypothesis that psoriasis may result from an inappropriate reaction to streptococci or other typically commensal bacteria colonizing the skin.

Our understanding of skin microbiota in psoriasis is currently limited. Previous studies have identified differences, albeit inconsistently, between healthy nonlesional skin and psoriatic lesions (Alekseyenko et al., 2013; Gao et al., 2008; Fahlén et al., 2012; Tett et al., 2017). Despite relatively small cohort sizes, aggregation of samples from various

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Abbreviations: OTU, operational taxonomic unit; PGA, Physician's Global Assessment; wUF, weighted UniFrac

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anatomical skin sites, and differences in sample type and sequencing methodology, some trends have been evident. Compared with nonlesional skin, aggregated psoriasis lesions trended towards decreased microbial diversity and differing relative abundance of resident skin bacteria, such as *Actinobacterium*, *Corynebacterium*, *Propionibacterium*, *Streptococcus*, and *Staphylococcus* (Alekseyenko et al., 2013; Gao et al., 2008; Fahlén et al., 2012).

These studies also did not focus on the microenvironment specificity, potential for site-specific trends, or longitudinal dynamics of skin microbiota in response to systemic therapy. For example, Alekseyenko et al. (2013) followed 17 individuals for 36 weeks and detected variations in skin microbiota that trended with treatment, but did not detect any significant changes from baseline. However, participants received a variety of treatments, and body-site specificity was not considered. More recently, Tett et al. (2017) employed metagenomic shotgun sequencing, which allows for multi-kingdom strain-level identification to analyze lesional and contralateral nonlesional ear and elbow sites. However, like the studies mentioned (Alekseyenko et al., 2013; Gao et al., 2008; Fahlén et al., 2012), their analysis in 28 individuals detected few discriminative features at the species level.

To address limitations of previous microbiome studies in plaque psoriasis, we sought to assess changes of the skin microbiome in the setting of a longitudinal phase 3b study of patients receiving up to 2 years of ustekinumab therapy (Blauvelt et al., 2017), by evaluating: (i) body-site-specific features of the skin microbiome in psoriasis; (ii) skin microbiome response to ustekinumab, a biologic therapy targeting interleukins 12 and 23, through week 112; and (iii) effects of disease recurrence and “response-based” maintenance dosing intervals on skin microbiota.

## RESULTS

### Patient characteristics and disposition

Among 478 patients with chronic plaque psoriasis who participated in the larger phase 3b study, a subset of 114 patients participated in this substudy examining skin microbiota composition prior to and during ustekinumab treatment. The main study, conducted to assess the efficacy and safety of extending the approved ustekinumab maintenance dosing interval beyond 12 weeks (Blauvelt et al., 2017), comprised two phases (Supplementary Figure S1 online, see Materials and Methods). In phase 1 (run-in), patients received 28 weeks of standardized open-label treatment with ustekinumab. In phase 2, patients achieving a therapeutic response (Physician’s Global Assessment [PGA] = 0/1) were randomized to receive every-12-week ustekinumab maintenance dosing (Group 1) or response-based dosing (every 12, 16, 20, or 24 week dosing determined by loss of PGA = 0/1) (Group 2) (Supplementary Figures S2 and S3 online). Baseline patient and disease characteristics were generally comparable among the overall and randomized substudy populations (Table 1).

### Cross-sectional analysis of lesional and nonlesional skin microbiome at baseline

We investigated the relationship between psoriasis and skin microbiota composition at baseline for each of six body sites (arm, axilla, buttock, leg, scalp, and trunk) selected for their

**Table 1. Baseline Patient and Disease Characteristics**

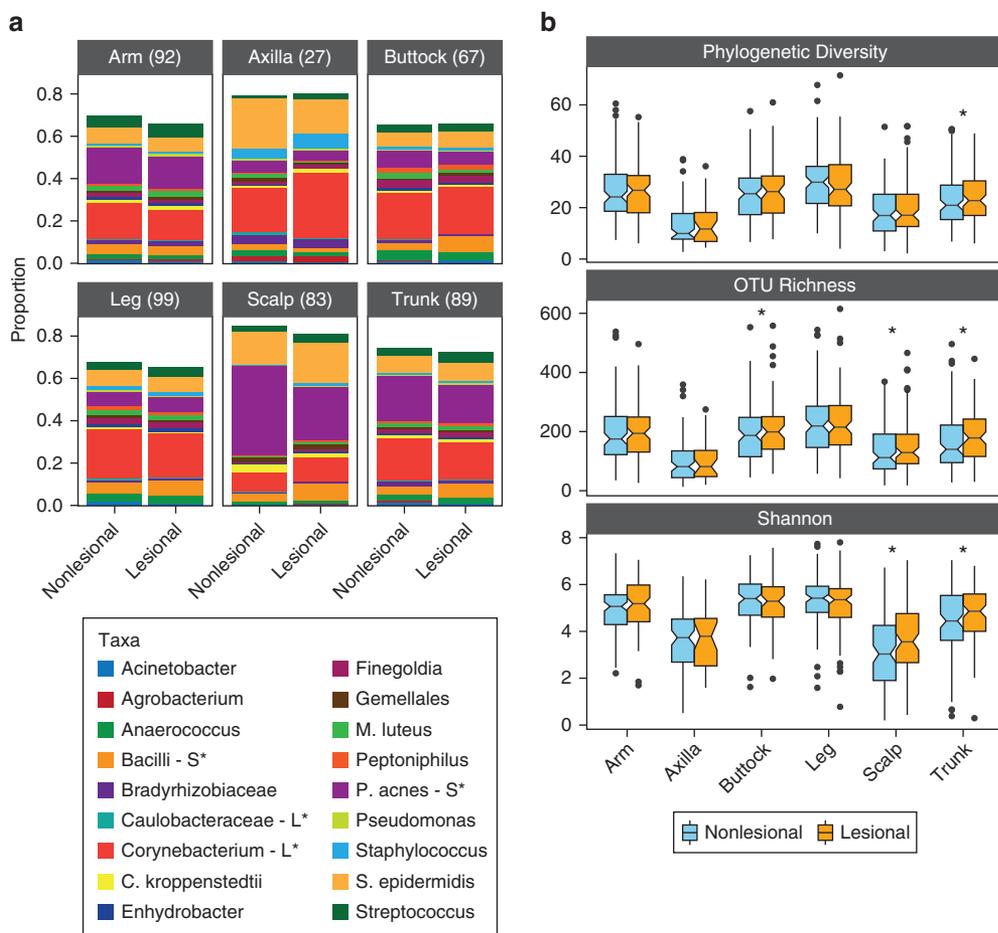
Characteristic	Phase 1	Phase 2
No. of patients	114	89
Age, y, mean	44.7	44.9
Body mass index, mean	30.6	31.1
Physician’s Global Assessment (0–5), mean	3.5	3.5
Psoriasis Area and Severity Index (0–72), mean	19.5	18.0
Body surface area, %, mean	24.4	21.6
Female, %	32	34
White, %	86	92
Hispanic, %	13	12
Smoker, %	49	51
Diabetic, %	9	10

predilection for psoriasis (see Materials and Methods for details of skin microbiota sampling). All analyses were site-specific, comparing lesional microbiota to that found on adjacent nonlesional skin for each patient. If no lesion was observed at a given body site, only nonlesional skin was sampled at each time point.

We first compared relative abundance of taxa at the species level or the highest level of taxonomic classification achieved. Nonlesional and lesional sites were remarkably similar, though some site-specific differences were detected (Figure 1a). In leg lesions, *Caulobacteraceae* and *Corynebacterium* were decreased in relative abundance versus nonlesional skin ( $P < 0.05$ ). In scalp lesions, *Bacilli* were increased and *Propionibacterium acnes* were decreased in relative abundance versus nonlesional skin ( $P < 0.05$ ). At the phylum level, lesional leg, scalp, and trunk carried more *Actinobacteria*, while *Firmicutes* were significantly lower in scalp and trunk lesions ( $P < 0.05$ ) versus adjacent nonlesional skin. Because *Streptococcus* throat infection has previously been associated with guttate psoriasis (Leung et al., 1995; McFadden et al., 2009; Telfer et al., 1992), we analyzed Spearman and Pearson correlations between *Streptococcus* relative abundance and disease severity as measured by PGA at baseline. While some weak correlations emerged, none withstood multiple test correction, suggesting that *Streptococcus* colonization of skin does not correlate with severity in lesional and non-lesional skin.

When comparing microbial diversity of lesional versus nonlesional skin, we identified body-site-specific differences (Figure 1b). The buttock, scalp, and trunk all exhibited increased operational taxonomic unit (OTU) richness in lesions ( $P < 0.05$ ); scalp and trunk lesions were more diverse by the Shannon diversity index ( $P < 0.05$ ). Trunk lesions had higher levels of phylogenetic diversity than nonlesional trunk skin ( $P < 0.05$ ).

We implemented a machine-learning approach to determine whether subtler variations in the skin microbiome distinguished lesional from nonlesional skin prior to treatment. For each body site, a random forest model was trained to identify lesional samples using exclusively OTU relative abundances. The accuracy of the models ranged from 56.4% to 87.8%. For arm, buttock, and trunk lesions, the classification accuracy was significantly better than chance (Table 2). The classification capacities of our models are



**Figure 1. The effect of the psoriatic lesions on skin microbiota at baseline.**

(a) Stacked bar plot depicting the mean relative presence of the most abundant taxa of both lesional and nonlesional skin by body site. Number of samples analyzed is indicated in parentheses on the top panel of the bar graph. Shown are the mean proportions of the top 14 taxa of a total of 18 taxa that were present in >1% of all samples. Significant differences are denoted by body site labels in the legend—arm, axilla, buttock, leg, scalp, and trunk. (b) Faceted boxplot of sample diversity for both lesional and nonlesional skin split by body site. Facets depict different  $\alpha$ -diversity metrics including phylogenetic diversity (top), operational taxonomic unit (OTU) richness (middle), and Shannon diversity (bottom). Upper and lower box hinges correspond to first and third quartiles, and the distance between these quartiles is defined as the interquartile range (IQR). Lines within the box depict median, and whiskers extend to the highest and lowest values within 1.5 times the IQR. Outliers of the IQR are depicted as dots above or below the whiskers. Significant ( $P < 0.05$ ) findings are denoted by asterisk.

similar to, or in some cases surpass, those previously reported (Statnikov et al., 2013), likely due to larger sample size and site specificity of training data. The ability of these models to classify samples as lesional or nonlesional suggests that there are subtle but reliable microbiome features that distinguish the two.

**Lesional and nonlesional skin microbiota respond similarly to ustekinumab**

We investigated changes in the microbiota of lesional and nonlesional skin resulting from systemic ustekinumab therapy by comparing relative abundances of major taxa over time at each body site, analyzing lesional and nonlesional sites separately. We detected significant changes in 11 major taxa

in  $\geq 1$  body site (Kruskal-Wallis test;  $P < 0.05$ , false discovery rate—adjusted), most of which were shared across >1 body site (Figure 2a). Lesional and nonlesional sites experienced similar changes in response to ustekinumab treatment; of the 41 findings of differential mean relative abundance, 22 were common to both lesional and nonlesional skin. Of those changes in taxa that were not shared across body sites, more changes in nonlesional sites than lesional sites (14 vs. 5 taxa, respectively) were observed (Figure 2b).

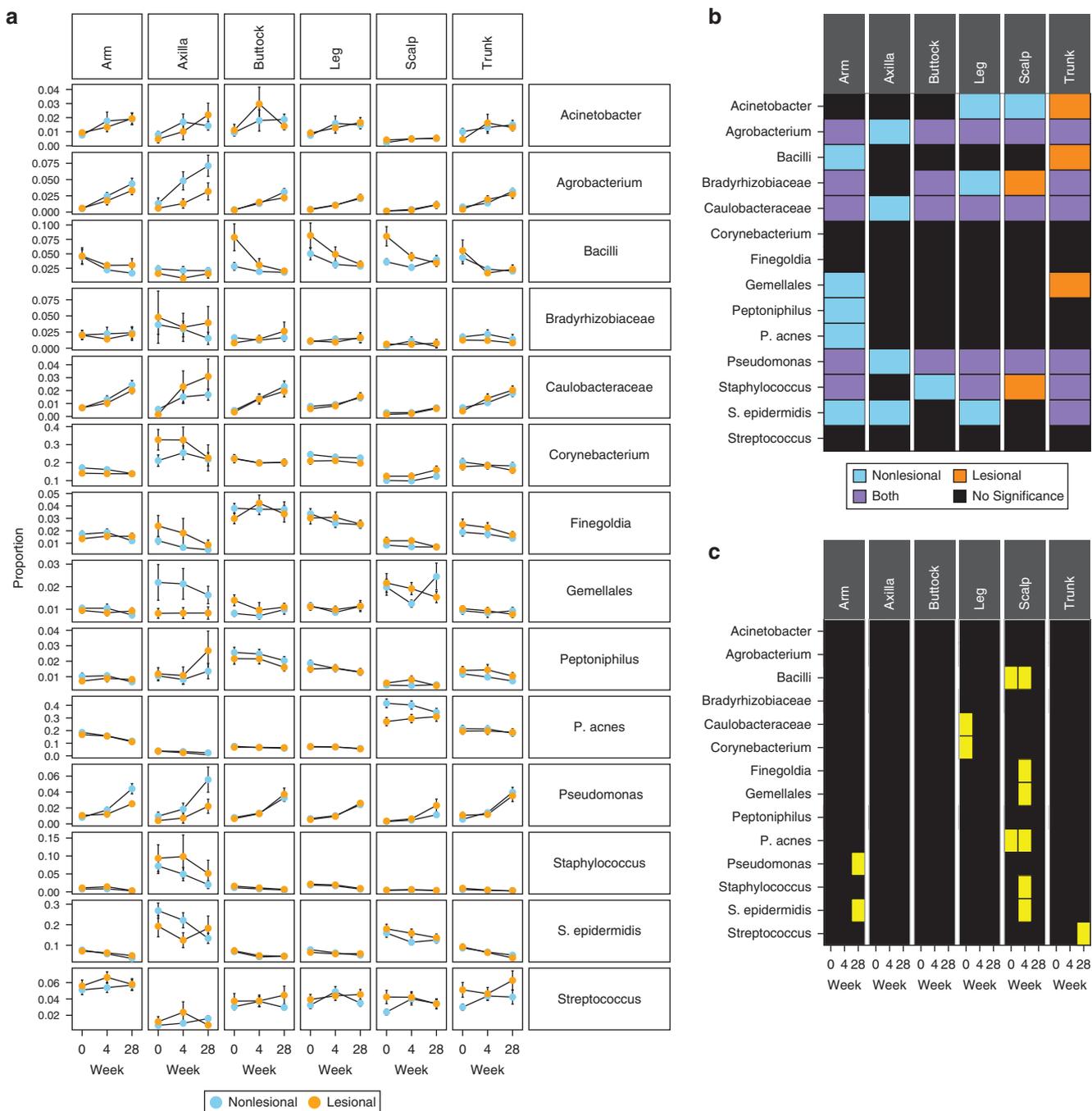
Because nonlesional skin experienced similar, if not greater, changes in specific bacterial taxa than lesional skin with ustekinumab treatment, we further investigated how much each site’s microbiome changed from baseline using weighted UniFrac (wUF) distances. The wUF metric, which ranges from 0 (complete similarity between the samples) to 1 (complete dissimilarity of samples being compared), assesses similarity between samples by incorporating both abundance and phylogenetic relationships of microbial communities (Lozupone et al., 2011). All body sites exhibited significant dissimilarity from their baseline sample, and ranged from 0.24 to 0.39 at week 4 and 0.29 to 0.45 at week 28 (Figure 3a). The leg and trunk both exhibited significantly higher rates of change in lesional skin at week 4 and week 28 ( $P < 0.05$ ) compared to non-lesional skin. Scalp remained the most stable of all body sites in both lesional and nonlesional skin.

To evaluate whether differences between lesional and nonlesional skin dissipated with ustekinumab therapy, we

**Table 2. Psoriatic Lesion Classification Accuracy by Body Site at Baseline**

Body Site	Accuracy, %	Area Under Curve	P-Value	Significance
Arm	87.8	0.895	8.30E-07	***
Axilla	70.8	0.547	4.20E-01	
Buttock	77.8	0.773	2.69E-02	*
Leg	60.5	0.675	1.43E-01	
Scalp	56.4	0.693	4.38E-01	
Trunk	78.1	0.868	3.79E-04	***

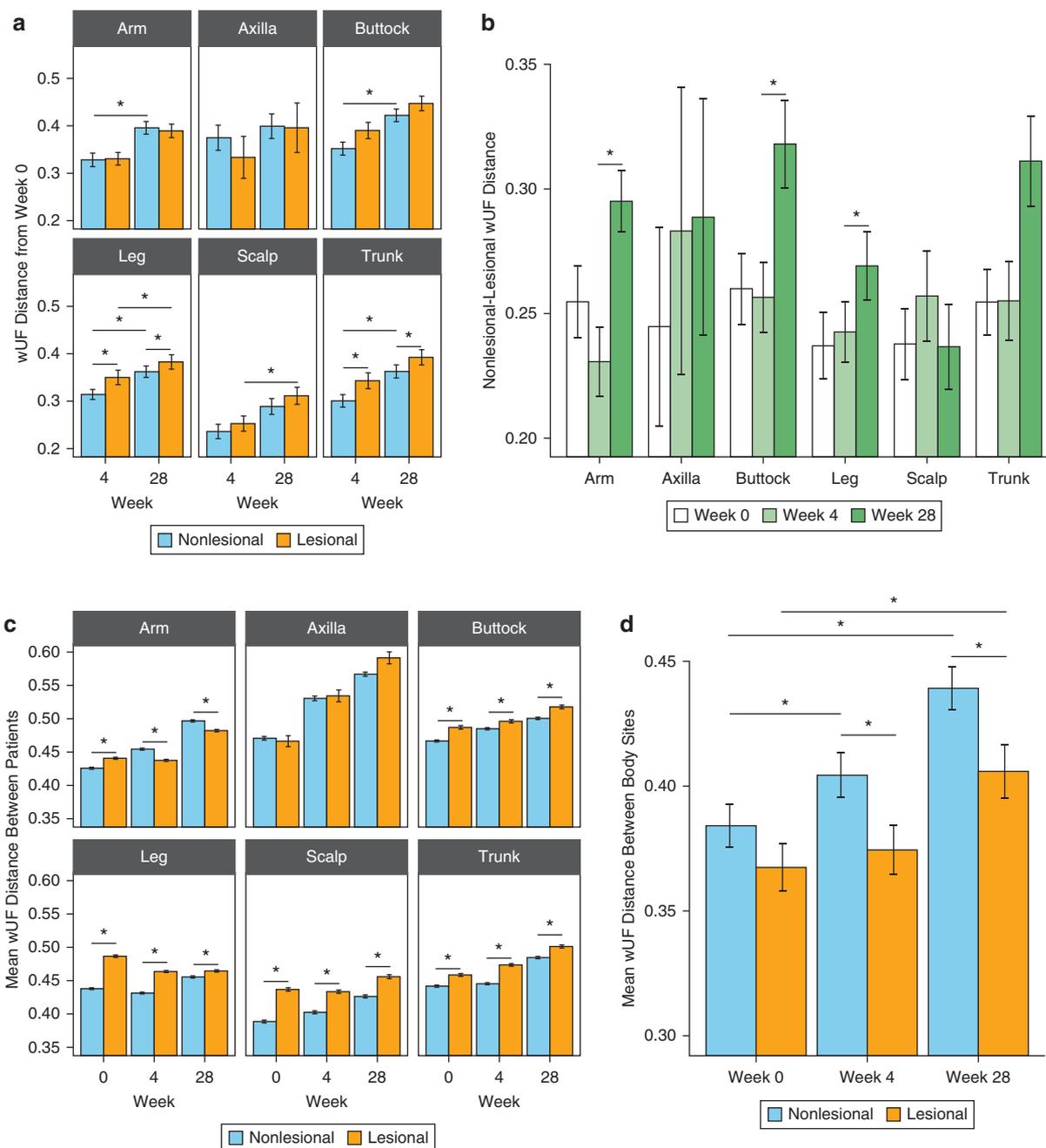
\* $P < 0.05$ .  
 \*\*\* $P < 0.001$ .



**Figure 2. Longitudinal changes in taxonomic composition following ustekinumab treatment.** (a) Plot shows the mean relative abundance for taxa identified as changing in at least one body site. Lesional and nonlesional skin are represented by orange and blue dots, respectively. Error bars represent the standard error of the mean. (b) Results of Kruskal-Wallis test for longitudinal changes in relative abundance levels for each of the major taxa by body site. Colored tiles represent significant findings in the nonlesional site only (blue), lesion site only (orange), or both sites (purple). (c) Results of paired-Wilcoxon testing differences between lesional and nonlesional sites at weeks 0, 4, and 28 by body site. Yellow tiles represent significant findings.

tested the persistence of the taxa that were differentially abundant at baseline. The baseline differences were no longer apparent by week 28; however, different taxa emerged to be significantly different at later time points between lesional and nonlesional skin (Figure 2c). No detectable differences in microbial diversity were observed by week 28, except for the trunk, where microbiota were more diverse in lesional versus nonlesional skin ( $P < 0.05$ ).

To evaluate whether the microbial communities from lesional and nonlesional skin had become more similar to each other with ustekinumab treatment, the wUF dissimilarity metric was employed to generate pairwise distances between lesional and nonlesional samples. Except for the scalp, all body sites exhibited similar trends towards divergence as treatment progressed, which was significant in the arm, buttock, and leg ( $P < 0.05$ ; Figure 3b), suggesting that lesional skin microbiota was not converging with nonlesional microbiota.



**Figure 3. Comparative microbiota dynamics of lesional and nonlesional skin during ustekinumab treatment.** (a) Lesional and nonlesional sites exhibit similar amounts of change during ustekinumab treatment. Bar plot showing the mean weighted UniFrac (wUF) distance between baseline (week 0) and subsequent visits (week 4 and week 28). Data are shown by body site. Nonlesional skin is represented by the blue bars, whereas lesional skin is represented by orange bars. Error bars depict the standard error of the mean. (b) Plot showing the mean wUF distance between paired lesional and nonlesional skin samples at multiple time points. Data are shown by body site. Error bars depict the standard error of the mean. Bar shading corresponds to time points. (c) Body sites variance and distinctness increase with ustekinumab treatment, as assessed by wUF distances between patients by body site and lesion status. Bars are colored by body site and shaded by lesion status. (d) Body site dispersion as measured by the mean wUF distances between body sites within patients. Bars are shaded by lesion status. Error bars depict the standard error of the mean. Significant ( $P < 0.05$ ) findings are denoted by asterisk.

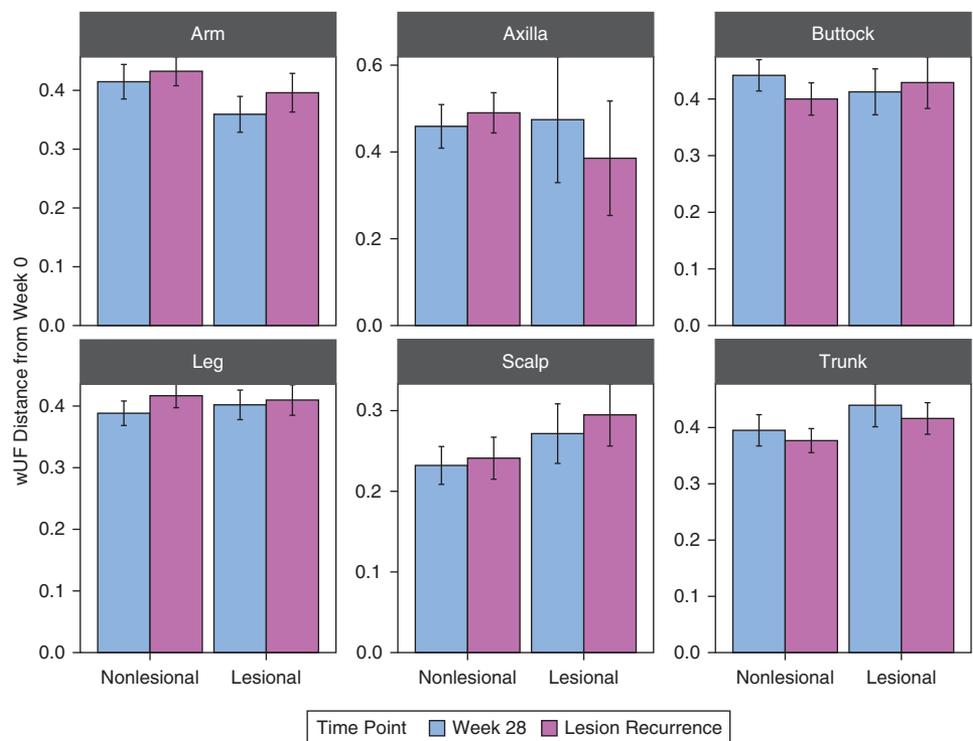
### Heterogeneity of skin microbiota within lesional versus nonlesional skin

To assess whether psoriasis may disrupt mechanisms maintaining body-site-specific microenvironments (eg, sebum or sweat production), intra-group heterogeneity of lesional and nonlesional skin was determined as the wUF distance between samples from different patients at the same body site. At baseline, heterogeneity observed in lesional skin was

greater than nonlesional skin across all body sites except the axilla ( $P < 0.05$ , Figure 3c). Apart from the arm, this pattern was maintained until week 28, though the magnitude of the difference diminished.

We next investigated whether body-site niches became more distinct with ustekinumab treatment by calculating the mean wUF distance between body sites per patient as a measure of body-site dispersion. At each time point,

**Figure 4. Recurrent lesions do not resemble prior lesions.** Weighted UniFrac (wUF) distances between the baseline samples and either remission (week 28) or recurrence samples. Distances were calculated for both lesional and nonlesional skin. Panels depict data by body site. Greater distances indicate greater dissimilarity. Error bars depict the standard error of the mean.



nonlesional skin exhibited greater dispersion than lesional skin ( $P < 0.05$ ), though this was not significant at baseline (Figure 3d). However, comparing lesional and nonlesional skin body sites, microbiota became more distinct from each other with treatment.

#### Effect of plaque recurrence and dosing regimen on the skin microbiome

For patients randomized to group 2 who achieved a PGA = 0/1 response, the week 28 ustekinumab dose was withheld until loss of response, that is, PGA  $\geq 2$  or up to 24 weeks after the previous dose at week 16. Note that in evaluating lesions, erythema, induration, and scaling were identified, but not scored, and resolution of all three was required for a lesion to be considered resolved. The response-based dose-interval determination design for the main study provided an opportunity to determine the pattern of skin microbiota composition with disease recurrence, as assessed by wUF distances between baseline (week 0) and either week 28 or the time of recurrence. No differences between the two comparisons (Figure 4) or between lesional and nonlesional skin were detected, supporting the concept of no specific pattern of skin microbiota composition within psoriasis lesions. Alternatively, the response of inflammation in psoriasis due to ustekinumab treatment may have effects on the skin microbiota that are not readily visible.

Using samples collected at the end of the study (week 112), after patients had received stable ustekinumab doses for  $\geq 70$  weeks, we investigated whether different maintenance dosing intervals impacted skin microbiota. No differences were detected between study maintenance dosing groups when comparing taxonomic composition at lesional and nonlesional skin sites. We also did not find any differences in

microbial diversity as measured by OTU richness, Shannon diversity, or phylogenetic diversity.

Using wUF distances to examine associations between skin microbiota and lesional or nonlesional skin (Supplementary Figure S4 online), we tested for nonrandom clustering in the maintenance dosing groups via body-site-specific Adonis models, which incorporated terms for lesion status and dosing group. We detected no differences for either term at any body site, suggesting that variations in ustekinumab maintenance dosing do not significantly affect the composition or diversity of skin microbial communities.

#### DISCUSSION

Here, we report a comprehensive analysis of the skin microbiome in psoriatic patients, including how it changed with ustekinumab treatment at standard and extended dosing intervals, in a body-site-specific manner. Prior to treatment, there were minor, body-site-specific differences in microbial diversity and composition when comparing lesional with nonlesional skin. Microbial heterogeneity was greater in lesional skin than nonlesional skin. During ustekinumab treatment, the composition of microbiota diverged further between lesional and nonlesional skin across body sites. Interestingly, the divergence observed between lesional and nonlesional skin during ustekinumab treatment varied by body site, perhaps indicating that inflammation in psoriasis affects the skin microbiome in a manner distinct from how it impacts the presence of physical plaques. These findings differ from those of previous studies of body-site-aggregated psoriasis lesions, which indicated trends towards decreased microbial diversity and differing relative abundance of resident skin bacteria in lesional versus nonlesional skin (Alekseyenko et al., 2013; Gao et al., 2008; Fahlén et al., 2012). Extending the ustekinumab dosing interval did not

affect the skin microbiome. Further still, when lesions recurred after extending the dosing interval, the skin microbiome did not return to its pretreatment status.

Our findings of body-site-specific changes in the skin microbiome and increasing variance with treatment argue for the existence of some “constraining” factor in psoriasis that limits the microbial diversity, which is relieved by therapy. A plausible “constraining” factor is the increased production of antimicrobial peptides in psoriasis, which could provide a strong selective pressure on the microbiota, limiting its composition to resistant bacteria. Ustekinumab significantly inhibits expression of antimicrobial peptides such as defensins and S100 proteins, which could remove the constraint on microbial variance (Brodmerkel et al., 2010). Other plausible constraining factors include structural differences in the skin due to abnormal epidermal proliferation or biochemical differences due to sebaceous gland atrophy and hair loss (Rittie et al., 2016).

We noted that as treatment progressed, the distinction between body sites increased, as measured by their mean wUF distance. Psoriatic lesions are known to impair certain homeostatic functions of the skin, such as sweat production (Cormia and Kuykendall, 1955; Johnson and Shuster, 1969; Rittie et al., 2016). This may hamper the ability of the skin to create distinct microenvironments, leading to greater similarity between body sites. As treatment progresses and lesions regress, these structures will begin to function more normally and perhaps increase the specificity of body-site niches. It has been documented that these functions return to normal gradually, over the course of at least 3 months (Suskind, 1954), which fits in with the timeline of our findings.

Although inherent in the main study design, the lack of a healthy control group for comparison limits this study. However, we noted an overall lower relative abundance of *Propionibacterium* and *Staphylococcus* and increased *Corynebacterium* in lesional skin when comparing to healthy skin microbiome data reported by Meisel et al. (2016), where the same sample collection, processing, and sequencing methods were utilized. Moreover, the combined proportion of rare taxa (<1% abundance) comprised a larger fraction of the psoriasis-associated microbial communities than is typical of nonlesional skin. These included taxa such as *Lactococcus*, *Porphyromonas*, *Paracoccus*, *Prevotella*, *Neisseria*, *Acinetobacter*, and *Fusobacterium*—taxa that are rarely identified as residents of human skin. This suggests that the skin of psoriatic patients may be less hospitable to typical skin bacteria and/or more susceptible to being colonized or contaminated by generally rare environmental bacteria. As an additional limitation, we did not differentiate lesion thickness or any gradation of clearing using a precise scoring system in classifying skin as nonlesional versus lesional. Furthermore, some subjects were on concomitant medications for other indications that may have the potential to alter the skin microbiome. It is also possible that differences exist at the strain level, or in fungal or viral components of the skin microbiota. Techniques such as metagenomic shotgun sequencing, as employed by Tett et al. (2017), could potentially resolve these differences.

Unlike atopic dermatitis, which is strongly associated with increases in *Staphylococcus aureus* and parallel decreases in microbial diversity (Kong et al., 2012), there is little consensus on specific bacterial taxa or diversity measures defining psoriasis. While this could owe to inconsistent sampling methods, analysis of aggregated samples from different body sites, and varying sequencing methodologies implemented by studies reported to date, it also could derive from the highly variable skin microbiota. Because of the low bioburden of bacteria on human skin and the high exposure to environmental contaminants (Ertl and Butte, 2012), the true relationship between psoriasis and the skin microbiome may elude even a large study such as ours.

In conclusion, the work presented here reaffirms and builds upon previous understandings of the skin microbiome and its interaction with psoriasis. Our work is distinguished from previous studies in the scale, attention to body-site-specific trends, standardized treatment, duration of follow-up, and longitudinal assessment as disease improved or recurred. These results, combined with future work from the same psoriasis cohort exploring the gut microbiome and response to ustekinumab, should inform future study design and may have medically relevant implications for diagnostics and therapeutics involving the skin microbiome.

## MATERIALS AND METHODS

### Substudy participants and design

Details of the main study ([ClinicalTrials.gov](https://clinicaltrials.gov/ct2/show/study?term=IDNCT01550744) IDNCT01550744; March 12, 2012), which was conducted at 42 US sites from March 2012 through July 2015, have been reported (Blauvelt et al., 2017). The main study was conducted according to the Declaration of Helsinki. Each site's Institutional Review Board approved the protocol; patients provided written informed consent. Inclusion and exclusion criteria are described elsewhere (Leonardi et al., 2008; Papp et al., 2008), where adults (aged 18–80 years) were required to have a diagnosis of moderate-to-severe plaque psoriasis ( $\geq 10\%$  body surface area, PGA score  $\geq 3$ ) for  $\geq 6$  months, but were excluded for confounding inflammatory disease, recent or known infection, malignancy, or prior anti-IL12 or IL23 therapy. Participation in the skin microbiome substudy was optional at a subset of 23 sites, and required additional informed consent. All substudy participants had skin swab samples collected before study agent administration and at a minimum of two follow-up visits (maximum of five visits). Beyond overall trial eligibility requirements, substudy participants had two of four body sites (arm, leg, scalp, and trunk) known to be prevalent disease areas represented with psoriatic plaques. Receipt of latent tuberculosis therapy and/or prohibited medications during specified time frames precluded participation. Substudy participation also required patients to abstain from showering; bathing; swimming; or using topical emollients, soaps, shampoos, deodorants, or other treatments and products for 24 hours prior to skin sampling. A summary of subjects on concomitant medications at the baseline visit is provided in [Supplementary Table S1](#) (online).

### Phase 1

Details of the timeline for the microbiome study are provided in [Supplementary Figure S1](#). The study's first phase followed patients' response to open-label ustekinumab treatment through 28 weeks. Patients received weight-based ustekinumab dosing (45 mg/90 mg for weight  $\leq 100$  kg/ $>100$  kg) at week 0, week 4, and week 16

(Blauvelt et al., 2017). Microbiome samples were taken at week 0, week 4, and week 28 at six body sites, including the arm, axilla, buttock, leg, scalp, and trunk. Skin swab samples were collected in a nonlesional area adjacent to the plaque lesion. Per the main trial design, patients achieving a therapeutic response (PGA score <2) at week 28 entered phase 2, while those who did not were discontinued from the study. Every effort was made to collect skin microbiome samples upon early discontinuation, though this was not always possible. In evaluating psoriatic lesions, erythema, induration, and scaling were identified, but not scored, and resolution of all three components was required for a lesion to be considered resolved. A sensitivity analysis showed that PGA scoring was representative of target lesion score.

### Phase 2

Also per the main trial design, during phase 2, the 89/114 substudy patients who achieved PGA score = 0/1 at week 28 were randomized 1:4 to group 1 (approved every-12-weeks maintenance) or Group 2 (every-12 to 24-weeks response-based dosing determined by time to loss of PGA = 0/1) (Supplementary Figure S1). Group 1 received the next ustekinumab dose according to the standard 12-week dosing regimen, while patients randomized to Group 2 entered the dose-interval determination period (week 28 to week 40) and did not receive ustekinumab until the next visit at which PGA score = 0/1 response was not maintained (ie, "recurrence" visit). PGA scores were assessed at week 32, week 36, and week 40, corresponding to 16, 20, and 24 weeks after the previous dose (week 28). Once a patient's psoriasis recurred, the next and subsequent ustekinumab doses were administered at the longest 4-week interval for which the patient had maintained response (PGA score <2). Thus, if patients experienced recurrence at week 32 (16 weeks after last dose), they received every-16-weeks maintenance dosing. If a patient's psoriasis did not recur by week 40 (24 weeks since last dose), they received every-24-weeks maintenance dosing.

Microbiome samples were collected from substudy participants at the "recurrence" time point. A final sample was collected at week 112 for all randomized patients, though ustekinumab treatment extended until week 124. At every visit, samples were taken from nonlesional skin at all 6 body sites. Lesional sites at baseline were also sampled for the remainder of the study, even if the plaque cleared.

### Data collection

Microbiome samples were collected using polyester-tipped applicators (#23-400-122; Fisher Scientific, Waltham, MA) moistened in sterile saline. Each clinical site was provided with a training video and a manual on how to collect the swabs. Negative controls swabs were taken by exposing the swab to the air and then submersing the swab in lysis buffer. DNA was extracted from swabs using the 96-well PureLink Pro 96 Genomic DNA kit (Invitrogen, Carlsbad, CA) per manufacturer's instructions, modified by adding 100  $\mu$ l phosphate-buffered saline to swabs pre-incubated in 300  $\mu$ l lysis buffer at the time of collection (vs. adding 400  $\mu$ l phosphate-buffered saline to dry swabs). Following extraction, DNA concentrations were quantitated fluorometrically using the Qubit instrument (Thermo Fisher Scientific, Waltham, MA).

### Sample sequencing and processing

The 16S rRNA gene V1-V3 region was amplified as previously described (Meisel et al., 2016). Resulting amplicons were sequenced using the Illumina MiSeq platform with paired-end 300 bp "V3"

chemistry, yielding 109,132,204 sequences, which were then processed with Quantitative Insights Into Microbial Ecology (Quantitative Insights Into Microbial Ecology 1.9.0; Caporaso et al., 2010).

Sequences were size filtered to be between 460 and 600 nucleotides, which resulted in 88,780,568 reads and a median of 13,120 reads per sample. Because of the dataset size, sequences were clustered into OTUs with a modified open-reference OTU picking method. The reference set was generated by randomly subsampling 1% of sequences and performing de novo OTU picking using an algorithm for clustering of unstructured data (Edgar, 2010) with a 97% sequence-similarity threshold. For each OTU, the most common sequence was selected as its representative sequence. This representative set was the reference for Quantitative Insights Into Microbial Ecology's default algorithm for clustering unstructured data, open-reference OTU picking script, `parallel_pick_otus_uclust_ref.py`. OTUs were assigned taxonomy using Ribosomal Database Project classifier (Cole et al., 2013) with the Greengenes 97% sequence-similarity database (DeSantis et al., 2006) Singletons, OTUs with only one sequence, were removed. OTUs corresponding to *Cyanobacteria* or *Delftia*, or unclassifiable, were removed as contaminants. This resulted in the removal of ~6.6% of OTUs, with a remaining 133,398 OTUs retained for subsequent analyses.

Samples were then subsampled to 2,000 sequences/sample for estimation of  $\alpha/\beta$  diversity metrics. Microbial diversity was estimated based on (i) number of observed OTUs (OTU richness); (ii) Shannon diversity; and (iii) Faith's phylogenetic diversity. Sample similarity was estimated using wUF (Lozupone et al., 2011)  $\beta$ -diversity metrics.

### Data analysis

Analyses were performed using the R statistical package (<https://www.r-project.org/>). Correlations between the relative abundances of *Streptococcus* with psoriasis severity was performed using both Pearson and Spearman tests. Comparisons of relative abundance, microbial diversity, or sample similarities were performed using Wilcoxon or Kruskal-Wallis tests where appropriate. *P*-value adjustments for multiple hypotheses testing were performed for the multiple taxonomic comparisons and testing between multiple time points by the false discovery rate method. Adjusted *P*-values <0.05 were considered significant. Calculation of nonmetric multidimensional scaling coordinates and ADONIS testing for nonrandom clustering was performed via the vegan Community Ecology Package software (<https://CRAN.R-project.org/package=vegan>). We used the *randomForest* package (<http://CRAN.R-project.org/doc/Rnews/>) in conjunction with Classification and Regression Training (<https://CRAN.R-project.org/package=caret>) to perform the machine learning analyses. The training set was created by randomly sampling 80% of patients; the remaining 20% were reserved for testing. The number of OTUs was pruned by first removing near-zero variance and highly correlated ( $\rho > 0.7$ ) OTUs. We then performed recursive feature elimination with subsets of OTUs of 10, 25, 50, 100, and all OTUs combined to identify the ideal number of features to include. The random forest parameter *mtry* was tuned over five iterations. Both recursive feature elimination and parameter tuning were evaluated by optimizing the receiver operating characteristics score >10-fold cross-validation repeated three times. Classification accuracy greater than chance was tested with a Bernoulli binomial test. Sequence data are publicly available from the National Center for Biotechnology Information's Short Read Archive, BioProject accession PRJNA427318.

**CONFLICTS OF INTERESTS**

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**SUPPLEMENTARY MATERIAL**

Supplementary material is linked to the online version of the paper at [www.jidonline.org](http://www.jidonline.org), and at <https://doi.org/10.1016/j.jid.2018.03.1501>.

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