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# The Surface Microbiome of Clinically Unaffected Skinfolks in Hidradenitis Suppurativa: A Cross-Sectional Culture-Based and 16S rRNA Gene Amplicon Sequencing Study in 60 Patients

Émeline Riverain-Gillet, Hélène Guet-Revillet, Jean-Philippe Jais, Marie-Noëlle Ungeheuer, Sabine Duchatelet, Maïa Delage, Thi Lam, Alain Hovnanian, Aude Nassif, Olivier Join-Lambert

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**THE SURFACE MICROBIOME OF CLINICALLY UNAFFECTED SKINFOLDS IN HIDRADENITIS SUPPURATIVA: A CROSS-SECTIONAL CULTURE BASED AND 16S rRNA GENE AMPLICON SEQUENCING STUDY IN 60 PATIENTS.**

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27 Olivier Join-Lambert, MD, PhD.  
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4 **THE SURFACE MICROBIOME OF CLINICALLY UNAFFECTED SKINFOLDS IN**  
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6 **HIDRADENITIS SUPPURATIVA: A CROSS-SECTIONAL CULTURE BASED AND**  
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8 **16S rRNA GENE AMPLICON SEQUENCING STUDY IN 60 PATIENTS.**  
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34 **Short title:** Skin dysbiosis in Hidradenitis Suppurativa  
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39 **Abbreviations used**  
40

41 BMI: body mass index  
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43 CoNS: coagulase negative staphylococci  
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45 FDR: false discovery rate  
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47 HF: hair follicle  
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49 HS: hidradenitis suppurativa  
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51 OTU: operational taxonomy unit  
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**ABSTRACT**

Hidradenitis Suppurativa (HS) is a chronic inflammatory disease of the skin associated with specific lesional dysbiotic features. We studied the microbiome of clinically unaffected typical HS sites (armpits, inguinal folds and gluteal clefts) in 60 HS patients and 17 healthy controls. 192 samples obtained by swabbing were analyzed by bacterial cultures. 116 randomly selected samples were studied by 16S rRNA gene amplicon sequencing. Patients and controls showed similar characteristics, except for smoking (87% vs 6%, respectively). HS skinfolds were characterized by an increased abundance of anaerobes, predominantly *Prevotella*, but also *Actinomyces*, *Campylobacter ureolyticus* and *Mobiluncus*, contrasting with a lower abundance of skin commensals such as *Staphylococcus epidermidis*, a major component of the skin microbiome, *Kocuria* and *Micrococcus luteus*. Three independent factors were associated with high anaerobes abundance by multivariate analysis: samples originating from HS patients ( $p=2.1 \times 10^{-4}$ ), body mass index ( $p=5 \times 10^{-5}$ ) and the sampling site, the gluteal cleft being the most anaerobic area, followed by inguinal folds and axilla ( $p=3 \times 10^{-6}$ ). The microbiome of clinically unaffected HS skinfolds is reminiscent, albeit to a minor extent, of the microbiome of chronic suppurative HS lesions and may fuel inflammation at a preclinical stage of the disease.

## INTRODUCTION

Hidradenitis suppurativa (HS) is a chronic inflammatory disease of the hair follicle (HF), characterized by recurrent or chronic nodules, abscesses and long lasting suppurative lesions, typically localized in the armpits, inguinal and perineal folds (Zouboulis et al., 2015). With an estimated prevalence of 1% in the general population, HS is a frequent disease with a high impact on quality of life (Janse et al., 2017).

HS pathophysiology is probably multifactorial, involving genetic factors (Pink et al., 2012), immune dysregulation (Hotz et al., 2016, Wolk et al., 2011), environmental and infectious factors (Nazary et al., 2011). 16S ribosomal RNA gene amplicon sequencing demonstrated that HS lesions are associated with anaerobic bacterial species with opportunistic pathogenic traits, which may fuel the inflammatory process (Guet-Revillet et al., 2014, Guet-Revillet et al., 2017, Ring et al., 2017, Schneider et al., 2019). In addition, HS keratinocytes display a pro-inflammatory phenotype with altered production of cytokines and dysregulation of antimicrobial peptide production which could alter the skin microbiome (Hotz et al., 2016).

Follicular hyperkeratosis, peri-folliculitis, occlusion and dilatation of the deepest portion of the HF are observed in more than 90% of early HS lesions (Boer and Weltevreden, 1996, Danby et al., 2013). These HF pathological features are also observed in clinically unaffected HS skinfolds. Associated with low-grade leucocytic infiltration, they demonstrate pre-clinical manifestations of the disease (van der Zee et al., 2012, Wortsman and Jemec, 2013). Recent studies performed in a limited number of patients suggested that the hair follicle and skin microbiome are altered in HS, depending on the HS area (Naik et al., 2019, Ring et al., 2017, Schneider et al., 2019). Postulating that skin dysbiosis may precede clinical signs of inflammation, we conducted a large comprehensive study of the microbiome of clinically unaffected skinfolds in HS, combining bacterial culture and 16S ribosomal RNA gene amplicon sequencing.

## RESULTS

**Subjects and samples.** 60 HS patients and 17 healthy controls subjects were prospectively enrolled in this study. They showed similar characteristics, except for a high prevalence of smokers in the HS group (87% vs 6%,  $p < 0.001$ , Table 1). Patients comprised 30 Hurley stage 1 (mild HS), 18 Hurley stage 2 (moderate HS) and 12 Hurley stage 3 (severe HS) patients, the patient's Hurley stage corresponding to the clinical severity of the most severe HS area (Hurley, 1989). 142 samples were obtained from patients and 50 from controls. The distribution of sample sites (armpits, inguinal folds and gluteal clefts) was similar in patients and controls.

**Bacterial culture results.** Similar bacterial species or groups were recovered from patients and controls. However, some taxa quantitatively differed between the two groups.

In controls' skinfolds, predominant bacterial populations were coagulase negative staphylococci (CoNS) (Table 2, Figure S1). *Staphylococcus epidermidis* was the most prevalent CoNS species and was recovered at similar rates from patients (70%) and controls (82%). However, the mean abundances of *S. epidermidis* and of *Staphylococcus hominis* were significantly lower in HS subjects' skinfolds (FDR = 0.03). Accordingly, the mean abundance of *Cutibacterium acnes* was lower in HS samples (FDR = 0.01) and a similar trend was observed for *Cutibacterium avidum* (FDR = 0.10).

On the other hand, the mean abundance of anaerobes was significantly higher in HS skinfolds (FDR: 0.002), and other anaerobic HS pathobionts such as *Actinomyces* spp and Milleri group streptococci were seldom cultured from HS skin samples, but never from control subjects.

Our next step was to assess microbiological variations according to HS sites and to the clinical severity of the disease. The mean abundance of anaerobes was significantly higher in inguinal folds and gluteal clefts (FDR = 0.001 and 0.01, respectively, Figure S1), but not in the armpits (FDR = 0.53). Contrarily, the decreased abundance of *C. acnes* was significant in the armpits (FDR =  $2.10^{-5}$ ), but not in other sites, and the lower abundance of *S. epidermidis* and *S. hominis*



1 abundances tended to be significant only in the inguinal folds (FDR = 0.05 and 0.09,  
2 respectively). Finally, the mean abundance of *Corynebacterium* spp correlated with a high  
3  
4 Hurley stage (FDR =  $4 \cdot 10^{-4}$ , Table S1). Conversely, the mean abundances of *Cutibacterium*  
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6  
7 spp and of *Actinomyces neuii* were lower in severe HS patients' skinfolds (FDR = 0.05).  
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10 Altogether, these data suggest that HS skinfolds are associated with a specific skin dysbiosis,  
11  
12 which differently varies according to the HS site and to the clinical severity of the disease.  
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18 **16S rRNA gene amplicon sequencing.** To refine these results, we performed 16S ribosomal  
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20 RNA gene sequencing on 116 randomly selected samples (79 samples from patients and 37  
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22 samples from controls, table 1 and Figure S2), allowing to identify 436 different bacterial taxa.  
23  
24 Bacterial richness was similar in the two sample groups (Figure 1a) but evenness was  
25  
26 significantly higher in HS samples, indicating a different distribution of bacterial taxa. At the  
27  
28 phylum level, *Firmicutes* and *Actinobacteria* were the most prevalent taxa both in HS and  
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30 controls' samples (Figure 1b). Considering the 10 top genera, a decreased relative abundance  
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32 of staphylococci (17% vs. 27%) was found in HS samples, confirming culture results.  
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37 Principal component analysis (PCA) showed that the two sample groups segregated on the first  
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39 component (horizontal axis, Figure 1c). Bacterial taxa that contributed to ecological distances  
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41 between HS patients' and controls' skinfolds microbiomes (Figure 1d) were one the first hand  
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43 staphylococci, and on the other hand anaerobes which have been previously associated with HS  
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45 lesions (Guet-Revillet et al., 2017).  
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48  
49 Univariate analysis performed on all samples showed that HS status, body mass index,  
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51 localization of samples and tobacco use correlated with a right shift of microbiomes on the first  
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53 component axis (Figure 2a and Figure S3). However, multivariate analysis demonstrated that  
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55 HS status ( $p = 2 \times 10^{-4}$ ), body mass index ( $p = 5 \times 10^{-5}$ ), and sampling site ( $p = 3 \times 10^{-6}$ ) but not  
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57 tobacco use ( $p = 0.72$ ) independently accounted for this differential distribution of samples  
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1  
2 Considering that 40/46 patients and 1/17 controls were smokers, this study was nevertheless  
3  
4 not powered to identify tobacco use as an independent factor associated with HS dysbiosis.

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6 The second component axis correlated with gender ( $p = 3 \times 10^{-8}$ , females being predominantly  
7  
8 associated with a higher abundance of *Lactobacillus* spp both in patients and controls, Table  
9  
10 S2), and with the clinical severity of the disease ( $p = 0.003$ ) (Figure 2b and Figure S3).  
11  
12 However, this last association was not significant after adjustment with gender ( $p = 0.52$ ).  
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15  
16 Our next step was to study the distribution of microbiomes among HS patients and controls by  
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18 hierarchical clustering analysis. The generated heatmap identified two clusters of samples  
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20 (Figure 3a and 3b). Cluster A samples were enriched in anaerobes, predominantly *Prevotella*,  
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22 *Porphyromonas*, *Dialister* and *Peptoniphilus asaccharolyticus*, whereas cluster B (aerobic  
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24 cluster) was associated with *Staphylococcus* and *Micrococcus* (Table S3). In agreement with  
25  
26 PCA results, 61% of HS samples were associated with the anaerobic cluster A (61% of samples,  
27  
28 Figure 3c), compared to only 22% of controls' ( $p=0.0002$ ).  
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32 Finally, 14 bacterial taxa statistically discriminated HS from control subjects' skin samples  
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34 ( $FDR \leq 0.05$ , Table 3, Figure 3d), including, at the genus and species level, four previously  
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36 identified anaerobic pathobionts of HS lesions (Guet-Revillet et al., 2017): predominantly  
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38 *Prevotella* ( $FDR=0.019$ ), but also *Actinomyces*, *Campylobacter ureolyticus* and *Mobiluncus*.  
39  
40 Contrarily, the relative abundance of major aerobic skin commensals including *S. epidermidis*,  
41  
42 *Micrococcus luteus* and *Kocuria* was lower in HS skinfolds. *Staphylococcus aureus* was the  
43  
44 sole bacterial species associated with the clinical severity of the disease ( $FDR = 0.03$ , Table  
45  
46 S4). Confirming culture results, *Corynebacterium* also tended to be associated with severe HS  
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48 ( $p= 2 \times 10^{-3}$ ,  $FDR= 0.09$ ).  
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51  
52 Altogether, these data suggest that the microbiome of clinically unaffected skinfolds in HS is  
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54 characterized by an anaerobic shift toward previously identified HS pathobionts, contrasting  
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56 with a decreased abundance of aerobic skin commensals, particularly *S. epidermidis*.  
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## DISCUSSION

The skin microbiome harbors a diversity of microorganisms that likely play an important role in skin homeostasis (Brestoff and Artis, 2013, Naik et al., 2015). There is increasing evidence suggesting that skin dysbiosis can promote skin inflammatory diseases. Skin dysbiosis frequently results from a dominant pathobiont such as *Cutibacterium acnes* in acne vulgaris (O'Neill and Gallo, 2018) or *Staphylococcus aureus* in atopic dermatitis (Chng et al., 2016, Nakatsuji and Gallo, 2019). In atopic dermatitis, *S. aureus* colonization precedes the onset of atopic flares, and the expansion of specific clones correlates with clinical severity (Paller et al., 2019). In HS, we previously showed that specific pathobionts are associated with HS lesions according to their clinical severity (Guet-Revillet et al., 2017). The aim of this work was to investigate the skin microbiome of clinically unaffected skinfolds in HS. Bacterial cultures were performed to identify bacteria to the species level which cannot always be obtained by 16S sequencing and to semi-quantify bacterial bioburden.

A major finding of this study is the decreased abundance of coagulase negative staphylococci (CoNS) of HS skinfolds and particularly of *Staphylococcus epidermidis*, that was quantitatively observed in bacterial cultures and confirmed by 16S sequencing. These data are in line with the reported low prevalence of coagulase negative staphylococci biofilms in the stratum corneum in HS (Ring et al., 2017). *S. epidermidis* is suspected to be a key guardian of the skin barrier (Nakamizo et al., 2015). It interacts with skin innate immune system and induces IL-17A1 CD81 T cells, that home to the epidermis and enhance innate barrier immunity by stimulating the production of antimicrobial peptides by keratinocytes, thus limiting the invasion of bacterial pathogens (Lai et al., 2010, Naik et al., 2015). The decreased abundance of *S. epidermidis* at the surface of HS skinfolds may participate in an altered skin barrier function in HS. A lower abundance of *Cutibacterium acnes* was observed by the culture method as previously reported

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2 (Naik et al., 2019, Schneider et al., 2019), contrasting with an increased relative abundance of  
3  
4 the *Propionibacteriaceae* family by 16S rRNA gene amplicon sequencing. Non-cultured  
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6 *Propionibacteriaceae* species likely account for this discrepancy.  
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8  
9 Contrarily, bacterial cultures and 16S rRNA sequencing demonstrated that anaerobes were  
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11 more prevalent in HS skinfolds than in controls, *Prevotella* being the most abundant anaerobic  
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13 taxon. *Prevotella* are anaerobic Gram-negative commensal rods of the oral and gut microbiome.  
14  
15 They show a particular ability to escape the immune system (Doke et al., 2017, Fteita et al.,  
16  
17 2018, Larsen, 2017) and are associated with a wide range of chronic infections, including  
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19 secondary skin infections and HS lesions (Brook, 2002, Guet-Revillet et al., 2014, Guet-  
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21 Revillet et al., 2017, Ring et al., 2017), chronic sinusitis (Brook, 2006), dental abscesses, and  
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23 dysbiotic inflammatory conditions such as periodontal disease (Hajishengallis, 2015, Robertson  
24  
25 and Smith, 2009). In our previous study on the microbiome of chronic HS lesions, *Prevotella*  
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27 relative abundance was significantly higher (15.8%), reflecting suppurative infectious  
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29 conditions (Guet-Revillet et al., 2017).  
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35 Our results indicate that a low relative abundance of *Prevotella* can be recovered from healthy  
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37 subjects' skinfolds, confirming previous studies on the human skin microbiome (Costello et al.,  
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39 2009). Compared to the dermis, some HF and sebaceous glands are moderately to severely  
40  
41 hypoxic in healthy subjects (Evans et al., 2006). The increased relative abundance of *Prevotella*  
42  
43 at the surface of HS skinfolds may therefore reflect an increased number of plugged hair follicle  
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45 in HS skin. In recent studies, *Prevotella* were associated with HS lesions, but not with normal  
46  
47 HS skinfolds (Naik et al., 2019, Ring et al., 2017, Schneider et al., 2019). This discrepancy may  
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49 be explained by different sampling methods, sampling site criteria or to environmental factors.  
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51  
52 Our results also showed that, in addition to HS, an increased body mass index and the gluteal  
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54 cleft site all contributed to an anaerobic shift of skin microbiomes. These findings are probably  
55  
56 due to the fact that maceration and surface anaerobic conditions are favored by overweight and  
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58 closed skinfolds. Contrarily to the results of a recent study (Naik et al., 2019), our data do not  
59  
60

1 support an association between the clinical severity of the disease and the relative abundance  
2 of anaerobes at the HS skin surface. This discrepancy may be explained by the low number of  
3 patients with mild HS in this study (2/12), or of confounding factors such as body mass index.  
4 However, *S. aureus* carriage on HS unaffected skinfolds was associated with the clinical  
5 severity of disease, as previously reported in HS lesional microbiomes (Guet-Revillet et al.,  
6 2017). Our results also suggest that HS clinical severity correlates with abundance of  
7 *Corynebacterium*, and inversely with that of *Cutibacterium* and *Actinomyces neuii* as  
8 previously reported (Naik et al., 2019, Schneider et al., 2019).

9 Finally, at the genus or species level, three other anaerobic opportunistic or pro-inflammatory  
10 taxa were associated with HS skinfolds: *Actinomyces*, *Mobiluncus*, and *Campylobacter*  
11 *ureolyticus* (previously *Bacteroides ureolyticus*). The genus *Actinomyces* is associated with a  
12 variety of chronic abscesses in humans, including HS lesions (Guet-Revillet et al., 2014, Guet-  
13 Revillet et al., 2017, Kononen and Wade, 2015). *Mobiluncus* spp are Gram variable anaerobic  
14 curved rods, closely linked to the genus *Actinomyces* (Lassnig et al., 1989). They were first  
15 associated with bacterial vaginosis and more recently with HS lesions (Guet-Revillet et al.,  
16 2017, Meltzer et al., 2008, Yeoman et al., 2013). *Campylobacter ureolyticus* is associated with  
17 other anaerobes in chronic periodontal disease and HS lesions (Guet-Revillet et al., 2017,  
18 Jumas-Bilak et al., 2009, Marchandin et al., 2010, Yu et al., 2016).

19 [A limitation of our study is that patients and controls likely differed according to hygiene habits,](#)  
20 [cosmetics use and previous antibiotic treatments. These environmental factors were not](#)  
21 [controlled and may therefore be confounders.](#) Importantly, 87% of patients were smokers,  
22 compared to 6% in controls, reflecting the high smoking rate in the general HS population  
23 (Daxhelet et al., 2016). Smoking is a well-recognized risk factor for HS (Akdogan et al., 2018,  
24 Revuz et al., 2008) but the link between smoking and HS is poorly understood. Cigarette  
25 smoking has been shown to alter oral and gut microbiomes (Karabudak et al., 2019, Savin et  
26 al., 2018, Wu et al., 2016). Our study as well as previous reports did not identify smoking as an

1  
2 independent factor associated with skin dysbiosis in HS (Ring et al., 2017, Schneider et al.,  
3  
4 2019). This issue needs to be clarified since these studies were not powered to demonstrate such  
5  
6 an association.  
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8  
9 To conclude, this study demonstrates that HS skinfolds are characterized by a decreased  
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11 abundance of major skin commensals, along with an increased abundance of anaerobes, and  
12  
13 that this anaerobic shift also depends on body mass index and localization. This dysbiosis is  
14  
15 reminiscent, but at a lower extent, of the microbiome of HS lesions (Guet-Revillet et al., 2017).  
16  
17 Whether HS skin dysbiosis simply reflects specific growth condition in occluded hair follicles,  
18  
19 results from and/or fuels low-grade HF inflammation in a vicious circle is an important issue to  
20  
21 solve. The non-invasive assessment of the skin microbiome in HS could be an interesting tool  
22  
23 to assess the risk of flares of the disease which are associated with an increased relative  
24  
25 abundance of *Prevotella* (Guet-Revillet et al., 2017) and to study the efficacy of medical  
26  
27 preventive treatments.  
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## 35 MATERIAL AND METHODS

36  
37 **Subjects.** This study was prospectively conducted between November 2011 and June 2014 at  
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39 the Centre d'Infectiologie Necker-Pasteur, a referral center for HS in Paris, France and at  
40  
41 ICAReB platform for the recruitment healthy controls. HS diagnosis was made by an  
42  
43 experienced physician using standard criteria (von der Werth et al., 2000). Main non-inclusion  
44  
45 criteria were age under 18 or over 60, pregnancy, cancer, congenital or acquired  
46  
47 immunosuppression, anti-inflammatory drugs or immunosuppressive treatments and  
48  
49 antibacterial treatment (systemic or topical) within the last month. This study was designed to  
50  
51 obtain real life data on the skin microbiome of HS patients. Patients and controls were advised  
52  
53 not to change their hygiene habits except for the use of deodorant on the day of the sampling  
54  
55 and topical antiseptics one week before inclusion.  
56  
57  
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1  
2 **Ethic committee approval.** This project was approved by the Ethical Committee “Comité de  
3  
4 Protection des Personnes Ile de France 2” (No IDRCB: 2011-A00536-35). All patients and  
5  
6 controls gave their written informed consent.  
7

8  
9 **Sampling methods.** We compared the microbiome from patients’ unaffected skinfolds in the  
10  
11 usual sites of HS lesion (armpits, gluteal cleft and inguinal folds) to those from healthy  
12  
13 volunteers. Samples were obtained by swabbing. For bacterial cultures, a 5 cm<sup>2</sup> surface was  
14  
15 sampled for 30 seconds using ESwabs (Copan Diagnostics, Brescia, Italy) that maintain the  
16  
17 viability of anaerobic and fastidious bacteria. Samples were transported to the Microbiology  
18  
19 Laboratory of the Necker-Enfants Malades Hospital at room temperature within 4 hours. For  
20  
21 16S rRNA gene sequencing, an adjacent 5 cm<sup>2</sup> surface was sampled for 1 minute using pre-  
22  
23 wetted Copan 167C swabs (Copan Diagnostics, Brescia, Italy) with ultrapure DNase free  
24  
25 distilled water (Invitrogen, Carlsbad, Ca, USA). Swabs were immediately discharged in sterile  
26  
27 water and stored at -80°C until DNA extraction.  
28  
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31  
32 **Bacterial cultures and identification methods.** Bacterial cultures were performed, analyzed  
33  
34 and semi-quantified as previously described (Guet-Revillet et al., 2017). 50 µl of the transport  
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36 medium were directly seeded on agar plates under aerobic (37 °C, 5% CO<sub>2</sub>) and anaerobic  
37  
38 conditions for 2 weeks. Anaerobic cultures were considered positive when the abundance or  
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40 diversity of the bacterial culture was increased under anaerobic conditions. Bacterial colonies  
41  
42 were semi-quantified using a scale ranging from 1 to 1000 colonies/sample (Guet-Revillet et  
43  
44 al., 2014, Guet-Revillet et al., 2017). A maximum of 10 different bacterial colonies per sample  
45  
46 was identified by MALDI-TOF mass spectrometry (Carbonnelle et al., 2010).  
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50  
51 **16S ribosomal RNA gene amplicon sequencing.** After thawing, samples were concentrated  
52  
53 to a final volume of 100 µL by centrifugation. Bacterial lysis was performed by adding  
54  
55 proteinase K to lysis buffer using the MagNa Pure LC DNA Isolation kit III (Roche Pharma,  
56  
57 Boulogne-Billancourt, France) and thermal shocks. DNA was extracted and amplified using the  
58  
59 eubacterial universal 16S primer set 27F/338R, targeting the V1 -V2 hypervariable regions of  
60

1  
2 the 16S ribosomal RNA gene (Fierer et al., 2008). DNA libraries were purified using Agencourt  
3  
4 AmPure XP beads (Agencourt Bioscience Corporation, Beverly, MA, USA) and sequenced  
5  
6 using 454 GS-FLX Titanium technology (Roche, Basel, Switzerland). We used QIIME  
7  
8 software (Version 1.6.0) for quality filtering of raw sequences, definition of operational  
9  
10 taxonomic units (OTUs) and taxonomic assignment. Chimeric sequences were excluded using  
11  
12 ChimeraSlayer (Edgar et al., 2011). Similar sequences were clustered into OTUs using Uclust,  
13  
14 with a minimum identity of 0.97. Representative sequences of each OTU were aligned and  
15  
16 taxonomy was assigned using the RDP classifier.  
17  
18

19  
20 **Statistical analysis.** All statistical analyses were performed using the R software v 3.5  
21  
22 (www.R-project.org). Comparison of bacterial culture profiles and bacterial culture abundance  
23  
24 was performed using the Fisher exact test and the Wilcoxon rank sum test, respectively.  
25  
26

27 Alpha diversity indexes, plots and analysis of variance were generated with the Phyloseq  
28  
29 package (McMurdie and Holmes, 2013) using non-normalized OTU counts. Hierarchical  
30  
31 ascendant clustering and principal component analysis (PCA) were performed after logarithmic  
32  
33 transformation of relative abundances using the pheatmap and ade4 package, respectively.  
34  
35 Bacterial taxa with a mean relative abundance below 0.4 % of the total number of reads obtained  
36  
37 from all samples were excluded for these analyses. PCA was performed on a relative taxon  
38  
39 abundance correlation matrix. A scree plot of eigenvalues was used to visually identify the  
40  
41 number of components explaining most of the variability in the data. To study bacterial taxa  
42  
43 contributing to ecological distances between microbiomes, both taxa and microbiomes were  
44  
45 projected on PCA ordination plots. Statistical comparisons of principal component coordinates  
46  
47 of microbiomes according to sample characteristics were computed by univariate and  
48  
49 multivariate linear models. Post-hoc subgroup comparison tests were adjusted by the Newman-  
50  
51 Keuls method.  
52  
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54  
55 Clustering of samples and taxa was performed on their respective distance matrix, based on  
56  
57 Pearson correlation. Dendrograms were generated using the Ward aggregation criterion.  
58  
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1  
2 Validity of clustering and number of centroids to choose for the data were determined using the  
3  
4 average silhouette score. Results were figured in a heatmap chart where samples and species  
5  
6 are re-ordered according to dendrogram.  
7

8  
9 To compare taxa relative abundance, we used a negative binomial regression model to take into  
10  
11 account overdispersion of taxa counts between samples (R Bioconductor edgeR package).  
12

13  
14 All statistical tests were considered as significant if the p-value was below 0.05. For multiple  
15  
16 comparisons procedures, p-values were adjusted by the false discovery rate (FDR) method.  
17  
18  
19

## 20 **DATA AVAILABILITY STATEMENT**

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22 Access to datasets related to this article will be available from September 2021 at Mendeley  
23  
24 data repository.  
25  
26  
27  
28

## 29 **CONFLICT OF INTEREST**

30  
31 The authors state no conflict of interest.  
32  
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35

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## 48 **CRedit statement**

49  
50 Émeline Riverain-Gillet: data curation, visualization, writing - original draft preparation  
51

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53 Hélène Guet-Revillet: investigation, writing - review and editing  
54

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56 Jean-Philippe Jais: methodology, resources, software, writing - review and editing  
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Sabine Duchatelet: writing - review and editing

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8 Aude Nassif: conceptualization, funding acquisition, writing - review and editing  
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10 Olivier Join-Lambert: conceptualization, funding acquisition, methodology, project  
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For Review Only

**REFERENCES**

- Akdogan N, Alli N, Uysal PI, Topcuoglu C, Candar T, Turhan T. Visfatin and insulin levels and cigarette smoking are independent risk factors for hidradenitis suppurativa: a case-control study. *Arch Dermatol Res* 2018;310(10):785-93.
- Boer J, Weltevreden EF. Hidradenitis suppurativa or acne inversa. A clinicopathological study of early lesions. *Br J Dermatol* 1996;135(5):721-5.
- Brestoff JR, Artis D. Commensal bacteria at the interface of host metabolism and the immune system. *Nat Immunol* 2013;14(7):676-84.
- Brook I. Secondary bacterial infections complicating skin lesions. *J Med Microbiol* 2002;51(10):808-12.
- Brook I. The role of anaerobic bacteria in sinusitis. *Anaerobe* 2006;12(1):5-12.
- Carbonnelle E, Mesquita C, Bille E, Day N, Dauphin B, Beretti JL, et al. MALDI-TOF mass spectrometry tools for bacterial identification in clinical microbiology laboratory. *Clin Biochem* 2010.
- Chng KR, Tay AS, Li C, Ng AH, Wang J, Suri BK, et al. Whole metagenome profiling reveals skin microbiome-dependent susceptibility to atopic dermatitis flare. *Nat Microbiol* 2016;1(9):16106.
- Costello EK, Lauber CL, Hamady M, Fierer N, Gordon JI, Knight R. Bacterial community variation in human body habitats across space and time. *Science* 2009;326(5960):1694-7.
- Danby FW, Jemec GB, Marsch W, von Laffert M. Preliminary findings suggest hidradenitis suppurativa may be due to defective follicular support. *Br J Dermatol* 2013;168(5):1034-9.

- 1  
2 Daxhelet M, Suppa M, Benhadou F, Djamei V, Tzellos T, Ingvarsson G, et al. Establishment  
3  
4 of a European Registry for hidradenitis suppurativa/acne inversa by using an open  
5  
6 source software. *J Eur Acad Dermatol Venereol* 2016;30(8):1424-6.  
7  
8  
9 Doke M, Fukamachi H, Morisaki H, Arimoto T, Kataoka H, Kuwata H. Nucleases from  
10  
11 *Prevotella intermedia* can degrade neutrophil extracellular traps. *Mol Oral Microbiol*  
12  
13 2017;32(4):288-300.  
14  
15  
16 Edgar RC, Haas BJ, Clemente JC, Quince C, Knight R. UCHIME improves sensitivity and  
17  
18 speed of chimera detection. *Bioinformatics* 2011;27(16):2194-200.  
19  
20  
21 Evans SM, Schrlau AE, Chalian AA, Zhang P, Koch CJ. Oxygen levels in normal and  
22  
23 previously irradiated human skin as assessed by EF5 binding. *J Invest Dermatol*  
24  
25 2006;126(12):2596-606.  
26  
27  
28 Fierer N, Hamady M, Lauber CL, Knight R. The influence of sex, handedness, and washing on  
29  
30 the diversity of hand surface bacteria. *Proc Natl Acad Sci U S A* 2008;105(46):17994-  
31  
32 9.  
33  
34  
35 Fteita D, Kononen E, Gursoy M, Ma X, Sintim HO, Gursoy UK. Quorum sensing molecules  
36  
37 regulate epithelial cytokine response and biofilm-related virulence of three *Prevotella*  
38  
39 species. *Anaerobe* 2018;54:128-35.  
40  
41  
42 Guet-Revillet H, Coignard-Biehler H, Jais JP, Quesne G, Frapy E, Poiree S, et al. Bacterial  
43  
44 pathogens associated with hidradenitis suppurativa, france. *Emerg Infect Dis*  
45  
46 2014;20(12):1990-8.  
47  
48  
49 Guet-Revillet H, Jais JP, Ungeheuer MN, Coignard-Biehler H, Duchatelet S, Delage M, et al.  
50  
51 The Microbiological Landscape of Anaerobic Infections in Hidradenitis Suppurativa: A  
52  
53 Prospective Metagenomic Study. *Clin Infect Dis* 2017;65(2):282-91.  
54  
55  
56 Hajishengallis G. Periodontitis: from microbial immune subversion to systemic inflammation.  
57  
58  
59  
60 *Nat Rev Immunol* 2015;15(1):30-44.

- 1  
2 Hotz C, Boniotto M, Guguin A, Surenaud M, Jean-Louis F, Tisserand P, et al. Intrinsic defect  
3  
4 in keratinocyte function leads to inflammation in Hidradenitis suppurativa. *J Invest*  
5  
6 *Dermatol* 2016.  
7  
8  
9 Hurley HJ. Axillary hyperhidrosis, apocrine bromhidrosis, hidradenitis suppurativa, and  
10  
11 familial benign pemphigus : surgical approach. In: Roenigk RK RH, editor. *Derm Surg.*  
12  
13 New-York: Marcel Dekker; 1989. p. 729-39.  
14  
15  
16 Janse IC, Deckers IE, van der Maten AD, Evers AWM, Boer J, van der Zee HH, et al. Sexual  
17  
18 health and quality of life are impaired in hidradenitis suppurativa: a multicentre cross-  
19  
20 sectional study. *Br J Dermatol* 2017;176(4):1042-7.  
21  
22  
23 Jumas-Bilak E, Roudiere L, Marchandin H. Description of 'Synergistetes' phyl. nov. and  
24  
25 emended description of the phylum 'Deferribacteres' and of the family  
26  
27 Syntrophomonadaceae, phylum 'Firmicutes'. *Int J Syst Evol Microbiol* 2009;59(Pt  
28  
29 5):1028-35.  
30  
31  
32 Karabudak S, Ari O, Durmaz B, Dal T, Basyigit T, Kalcioglu MT, et al. Analysis of the effect  
33  
34 of smoking on the buccal microbiome using next-generation sequencing technology. *J*  
35  
36 *Med Microbiol* 2019.  
37  
38  
39 Kononen E, Wade WG. Actinomyces and related organisms in human infections. *Clin*  
40  
41 *Microbiol Rev* 2015;28(2):419-42.  
42  
43  
44 Lai Y, Cogen AL, Radek KA, Park HJ, Macleod DT, Leichtle A, et al. Activation of TLR2 by  
45  
46 a small molecule produced by *Staphylococcus epidermidis* increases antimicrobial  
47  
48 defense against bacterial skin infections. *J Invest Dermatol* 2010;130(9):2211-21.  
49  
50  
51 Larsen JM. The immune response to *Prevotella* bacteria in chronic inflammatory disease.  
52  
53 *Immunology* 2017;151(4):363-74.  
54  
55  
56 Lassnig C, Dorsch M, Wolters J, Schaber E, Stoffler G, Stackebrandt E. Phylogenetic evidence  
57  
58 for the relationship between the genera *Mobiluncus* and *Actinomyces*. *FEMS Microbiol*  
59  
60 *Lett* 1989;53(1-2):17-21.

- 1  
2 Marchandin H, Damay A, Roudiere L, Teyssier C, Zorogniotti I, Dechaud H, et al. Phylogeny,  
3  
4 diversity and host specialization in the phylum Synergistetes with emphasis on strains  
5  
6 and clones of human origin. *Res Microbiol* 2010;161(2):91-100.  
7  
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9  
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56  
57  
58  
59  
60
- McMurdie PJ, Holmes S. phyloseq: an R package for reproducible interactive analysis and graphics of microbiome census data. *PLoS One* 2013;8(4):e61217.
- Meltzer MC, Desmond RA, Schwebke JR. Association of *Mobiluncus curtisii* with recurrence of bacterial vaginosis. *Sex Transm Dis* 2008;35(6):611-3.
- Naik HB, Jo JH, Paul M, Kong HH. Skin microbiota perturbations are distinct and disease severity-dependent in hidradenitis suppurativa. *J Invest Dermatol* 2019.
- Naik S, Bouladoux N, Linehan JL, Han SJ, Harrison OJ, Wilhelm C, et al. Commensal-dendritic-cell interaction specifies a unique protective skin immune signature. *Nature* 2015;520(7545):104-8.
- Nakamizo S, Egawa G, Honda T, Nakajima S, Belkaid Y, Kabashima K. Commensal bacteria and cutaneous immunity. *Seminars in immunopathology* 2015;37(1):73-80.
- Nakatsuji T, Gallo RL. The role of the skin microbiome in atopic dermatitis. *Ann Allergy Asthma Immunol* 2019;122(3):263-9.
- Nazary M, van der Zee HH, Prens EP, Folkerts G, Boer J. Pathogenesis and pharmacotherapy of Hidradenitis suppurativa. *Eur J Pharmacol* 2011;672(1-3):1-8.
- O'Neill AM, Gallo RL. Host-microbiome interactions and recent progress into understanding the biology of acne vulgaris. *Microbiome* 2018;6(1):177.
- Paller AS, Kong HH, Seed P, Naik S, Scharschmidt TC, Gallo RL, et al. The microbiome in patients with atopic dermatitis. *The Journal of allergy and clinical immunology* 2019;143(1):26-35.
- Pink AE, Simpson MA, Desai N, Dafou D, Hills A, Mortimer P, et al. Mutations in the gamma-secretase genes NCSTN, PSENEN, and PSEN1 underlie rare forms of hidradenitis suppurativa (acne inversa). *J Invest Dermatol* 2012;132(10):2459-61.

- 1  
2 Revuz JE, Canoui-Poitrine F, Wolkenstein P, Viallette C, Gabison G, Pouget F, et al.  
3  
4 Prevalence and factors associated with hidradenitis suppurativa: results from two case-  
5  
6 control studies. *J Am Acad Dermatol* 2008;59(4):596-601.  
7  
8  
9 Ring HC, Thorsen J, Saunte DM, Lilje B, Bay L, Riis PT, et al. The Follicular Skin Microbiome  
10  
11 in Patients With Hidradenitis Suppurativa and Healthy Controls. *JAMA Dermatol*  
12  
13 2017;153(9):897-905.  
14  
15  
16 Robertson D, Smith AJ. The microbiology of the acute dental abscess. *J Med Microbiol*  
17  
18 2009;58(Pt 2):155-62.  
19  
20 Savin Z, Kivity S, Yonath H, Yehuda S. Smoking and the intestinal microbiome. *Arch*  
21  
22 *Microbiol* 2018;200(5):677-84.  
23  
24  
25 Schneider AM, Cook LC, Zhan X, Banerjee K, Cong Z, Imamura-Kawasawa Y, et al. Loss of  
26  
27 skin microbial diversity and alteration of bacterial metabolic function in Hidradenitis  
28  
29 Suppurativa. *J Invest Dermatol* 2019.  
30  
31  
32 van der Zee HH, de Ruyter L, Boer J, van den Broecke DG, den Hollander JC, Laman JD, et al.  
33  
34 Alterations in leucocyte subsets and histomorphology in normal-appearing perilesional  
35  
36 skin and early and chronic hidradenitis suppurativa lesions. *Br J Dermatol*  
37  
38 2012;166(1):98-106.  
39  
40  
41 von der Werth JM, Williams HC, Raeburn JA. The clinical genetics of hidradenitis suppurativa  
42  
43 revisited. *Br J Dermatol* 2000;142(5):947-53.  
44  
45  
46 Wolk K, Warszawska K, Hoeflich C, Witte E, Schneider-Burrus S, Witte K, et al. Deficiency  
47  
48 of IL-22 contributes to a chronic inflammatory disease: pathogenetic mechanisms in  
49  
50 acne inversa. *J Immunol* 2011;186(2):1228-39.  
51  
52  
53 Wortsman X, Jemec G. A 3D ultrasound study of sinus tract formation in hidradenitis  
54  
55 suppurativa. *Dermatol Online J* 2013;19(6):18564.  
56  
57  
58 Wu J, Peters BA, Dominianni C, Zhang Y, Pei Z, Yang L, et al. Cigarette smoking and the oral  
59  
60 microbiome in a large study of American adults. *ISME J* 2016;10(10):2435-46.

1  
2 Yeoman CJ, Thomas SM, Miller ME, Ulanov AV, Torralba M, Lucas S, et al. A multi-omic  
3  
4 systems-based approach reveals metabolic markers of bacterial vaginosis and insight  
5  
6 into the disease. *PLoS One* 2013;8(2):e56111.  
7

8  
9 Yu XL, Chan Y, Zhuang LF, Lai HC, Lang NP, Lacap-Bugler DC, et al. Distributions of  
10  
11 Synergistetes in clinically-healthy and diseased periodontal and peri-implant niches.  
12  
13 *Microb Pathog* 2016;94:90-103.  
14

15  
16 Zouboulis CC, Desai N, Emtestam L, Hunger RE, Ioannides D, Juhasz I, et al. European S1  
17  
18 guideline for the treatment of hidradenitis suppurativa/acne inversa. *J Eur Acad*  
19  
20 *Dermatol Venereol* 2015;29(4):619-44.  
21  
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23  
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26  
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**Table 1. Subjects and samples**

Characteristics of subjects	HS patients	Controls	P
Number of subjects	60	17	
Gender M/F (sex ratio)	29/31 (0.94)	7/10 (0.70)	0.78 <sup>1</sup>
Age, years, mean (SD)	34.7(10.6)	36.6 (10.0)	0.5 <sup>2</sup>
BMI (kg.m <sup>-2</sup> ), mean (SD)	25.7 (5.0)	24.5 (2.7)	0.37 <sup>1</sup>
Clinical severity of HS			
Hurley stage 1, n (%)	30 (50)	na	
Hurley stage 2, n (%)	18 (30)	na	
Hurley stage 3, n (%)	12 (20)	na	
Familial HS, n (%)	25 (42)	na	
Smokers, n (%)	52 (87)	1 (6)	< 0.001 <sup>1</sup>
Bacterial culture samples			
Number of subjects	60	17	
Number of samples	142	50	
Clinical severity of patients			
Hurley stage 1, n of samples (%)	87 (61)	na	
Hurley stage 2, n of samples (%)	40 (28)	na	
Hurley stage 3, n of samples (%)	15 (11)	na	
Samples localization			
Armpits, n (%)	51 (36)	17 (34)	0.97 <sup>1</sup>
Inguinal folds, n (%)	47 (33)	17 (34)	
Gluteal cleft, n (%)	44 (31)	16 (32)	
16S rRNA gene amplicon sequencing samples			
Number of subjects	46	17	
Number of samples	79	37	
Age, years, mean (SD)	33.4 (10.2)	36.6 (10.0)	0.46 <sup>2</sup>
Gender M/F (sex ratio)	22/24 (0.92)	7/10 (0.7)	0.78 <sup>1</sup>
BMI, kg.m <sup>-2</sup> , mean (SD)	25.4 (4.6)	24.5 (2.7)	0.53 <sup>2</sup>
Familial HS, n (%)	20 (43)	na	
Smokers, n (%)	40 (87)	1 (6)	< 0.001 <sup>1</sup>
Clinical severity of patients			
Hurley stage 1, n of samples (%)	40 (51)	na	
Hurley stage 2, n of samples (%)	28 (35)	na	
Hurley stage 3, n of samples (%)	11 (14)	na	
Samples localization			
Armpits, n (%)	24 (30)	11 (30)	0.93 <sup>1</sup>
Inguinal folds, n (%)	32 (41)	14 (38)	
Gluteal cleft, n (%)	23 (29)	12 (32)	

<sup>1</sup> Fisher exact test (X2)<sup>2</sup> unpaired t-test

na:

not

attributable

**Table 2. Bacterial culture results of HS skinfolds compared to controls**

Bacterial species or groups	HS patients (142 samples)		Control subjects (50 samples)		P	FDR
	F	mA	F	mA		
<i>S. aureus</i>	8	38	2	2	0.12	0.22
<b><i>S. epidermidis</i><sup>2</sup></b>	<b>70</b>	<b>334</b>	<b>82</b>	<b>518</b>	<b>0.01</b>	<b>0.03</b>
<b><i>S. hominis</i><sup>2</sup></b>	<b>22</b>	<b>101</b>	<b>42</b>	<b>178</b>	<b>0.01</b>	<b>0.03</b>
<i>S. haemolyticus</i>	21	121	18	104	0.66	0.72
<i>S. lugdunensis</i>	25	82	12	30	0.05	0.12
<b>Other CoNS species<sup>2</sup></b>	<b>15</b>	<b>59</b>	<b>34</b>	<b>156</b>	<b>0.003</b>	<b>0.02</b>
<b><i>Cutibacterium acnes</i><sup>2</sup></b>	<b>1</b>	<b>7</b>	<b>12</b>	<b>49</b>	<b>0.001</b>	<b>0.01</b>
<i>Cutibacterium avidum</i>	25	58	36	205	0.04	0.10
<i>Cutibacterium</i> spp	8	9	4	10	0.37	0.50
<i>C. tuberculostearicum</i>	35	170	40	214	0.42	0.53
<i>Corynebacterium</i> spp	52	426	38	135	0.02	0.06
<i>Dermabacter hominis</i>	44	194	48	214	0.69	0.72
Micrococcaceae	16	36	30	16	0.05	0.12
Enterobacteriaceae	30	143	18	43	0.09	0.19
<i>Enterococcus</i> spp	27	113	18	76	0.20	0.31
<i>Streptococcus agalactiae</i>	6	52	4	21	0.53	0.63
<i>Actinomyces neuii</i>	15	69	14	66	0.84	0.84
<i>Actinomyces</i> spp	2	7	0	0	0.30	0.44
Milleri group streptococci	4	18	0	0	0.18	0.30
<b>Anaerobes<sup>1</sup></b>	<b>53</b>	<b>273</b>	<b>32</b>	<b>21</b>	<b>10<sup>-4</sup></b>	<b>0.002</b>

F: frequency (% of positive samples), mA= mean abundance (number of colonies/sample).

CoNS: coagulase negative staphylococci, FDR: false discovery rate. Taxa in bold: significant mean abundance difference between the two samples groups (FDR  $\leq$  0.05). <sup>1</sup> taxa statistically associated with HS patients' unaffected skinfolds. <sup>2</sup> taxa statistically associated with control subjects' skinfolds.

**Table 3. Bacterial taxa differentially associated with HS and control subjects' skinfolds as assessed by 16S rRNA gene amplicon sequencing**

**Bacterial taxa associated with HS patients' skinfolds**

[Phylum] Taxa	HS Patients (79 samples)		Controls (37 samples)		P	FDR
	F	mA	F	mA		
[Bact] [g] <i>Prevotella</i>	80	1.62	41	0.28	4.10 <sup>-4</sup>	0.019
[Act] [g] <i>Actinomyces</i>	25	0.07	5	1.10 <sup>-5</sup>	2.10 <sup>-4</sup>	0.013
[Act] [g] <i>Mobiluncus</i>	33	0.05	0	0	3.10 <sup>-7</sup>	2.10 <sup>-4</sup>
[Pro] [s] <i>Campylobacter ureolyticus</i>	63	0.43	22	0.03	2.10 <sup>-6</sup>	4.10 <sup>-4</sup>
[Act] [f] <i>Propionibacteriaceae</i>	41	0.26	32	0.01	2.10 <sup>-4</sup>	0.013
[Fir] [s] <i>Streptococcus agalactiae</i>	19	0.20	3	5.10 <sup>-6</sup>	7.10 <sup>-4</sup>	0.027
[Syn] [f] <i>Dethiosulfovibrionaceae</i>	20	0.03	0	0	9.10 <sup>-5</sup>	0.007
[Syn] [g] <i>Pyramidobacter</i>	14	0.02	0	0	2.10 <sup>-3</sup>	0.046
[Pro] [f] <i>Oxalobacteraceae</i>	80	3.73	70	0.52	4.10 <sup>-4</sup>	0.019

**Bacterial taxa associated with control subjects' skinfolds**

[Phylum] Taxa	HS patients (79 samples)		Controls (37 samples)		P	FDR
	F	mA	F	mA		
[Fir] [s] <i>Staphylococcus epidermidis</i>	99	13	97	35	3.10 <sup>-4</sup>	0.015
[Act] [s] <i>Micrococcus luteus</i>	51	0.23	73	0.91	0.001	0.036
[Act] [g] <i>Kocuria</i>	14	0.01	41	0.19	7.10 <sup>-5</sup>	0.007
[Pro] [g] <i>Vibrio</i>	1	0.0003	14	0.01	5.10 <sup>-4</sup>	0.021
[Pro] [g] <i>Rhodanobacter</i>	0	0	16	0.03	4.10 <sup>-6</sup>	0.001

F: frequency (% of positive samples), mA= mean abundance (%). Act: *Actinobacteria*, Bact: *Bacteroidetes*, Fir: *Firmicutes*, Pro: *Proteobacteria*, Syn: *Synergistetes*, [f]: family, [g]: genus, [s]: species, FDR: false discovery rate.

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2 **Figure 1 title: Microbiomes of Hidradenitis Suppurativa clinically unaffected skinfolds as**  
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4 **assessed by 16S rRNA gene amplicon sequencing**  
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9 **Figure 1 legend:**  
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11 a: alpha-diversity metrics of HS (Hidradenitis Suppurativa) and control samples microbiomes.

12 Box plots represent the 25%–75% interquartile range and the median.  
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14 b: main bacterial phyla and top ten taxa (genus level) in HS and control samples.  
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16 c: ordination plots of all studied microbiomes (HS and controls). Each dot represents a  
17 microbiome. Computed centroids (mean values) are presented. Inertia ellipses correspond to  
18 two thirds of samples.  
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25 d: bacterial taxa accounting for ecological distances.  
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2 **Figure 2 title: Variations of skinfolds microbiomes according to sample type (HS vs**  
3 **controls), body mass index, skin area, tobacco use, gender and clinical severity of HS.**  
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7 **Figure 2 legend**  
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9 a: distribution of microbiomes on the first component axis (PC1) according to sample type (HS  
10 vs controls), body mass index, skin area and tobacco use (horizontal line: median, box: IQR).  
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12 Clinical factors without significant variations on the first component axis of the ordination plot  
13 are not presented. Corresponding ordination plots: see figure S2.  
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18 b: distribution of microbiomes on the second component axis according to sample type (HS vs  
19 controls), gender and clinical severity of HS (horizontal line: median, box: IQR). Corresponding  
20 ordination plots: see figure S2 Clinical factors without significant variations on the 2<sup>nd</sup>  
21 component axis of the ordination plot are not presented.  
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24  
25 c. Results of univariate (Univar) and multivariate (Multivar) analysis.  
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28 Smk: smokers' samples, NSmk: non-smokers' samples, BMI: kg. m<sup>-2</sup>, ND: not done.  
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2 **Figure 3 title: Skin microbiome profiles and taxa associated with HS skinfolds**  
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5 **Figure 3 legend**  
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8 a. Hierarchical clustering of all samples according to microbiome compositions. On the  
9 heatmap, columns represent samples and lines represent taxa. 2 clusters of samples were  
10 identified. Cluster “A”: anaerobic profile (horizontal cluster 2). Cluster “B”: aerobic profile  
11 (horizontal cluster 1).  
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16 b. Representation of cluster A and B samples on the PCA ordination plot.  
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18

19 c. Repartition of anaerobic (cluster A) and aerobic (cluster B) microbiomes among patients and  
20 control samples.  
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23 c. Bacterial taxa differentially associated with HS or control subjects' skinfolds (FDR  $\leq$  0.05).  
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25 Act: *Actinobacteria*, Bact: *Bacteroidetes*, Fir: *Firmicutes*, Fus: *Fusobacteria*, Pro:  
26 *Proteobacteria*. [f]: family, [g]: genus, [s]: species. \*: bacterial taxa statistically associated with  
27 HS patients' unaffected skinfolds, +: bacterial taxa statistically associated with control subjects'  
28 skinfolds.  
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Figure 1

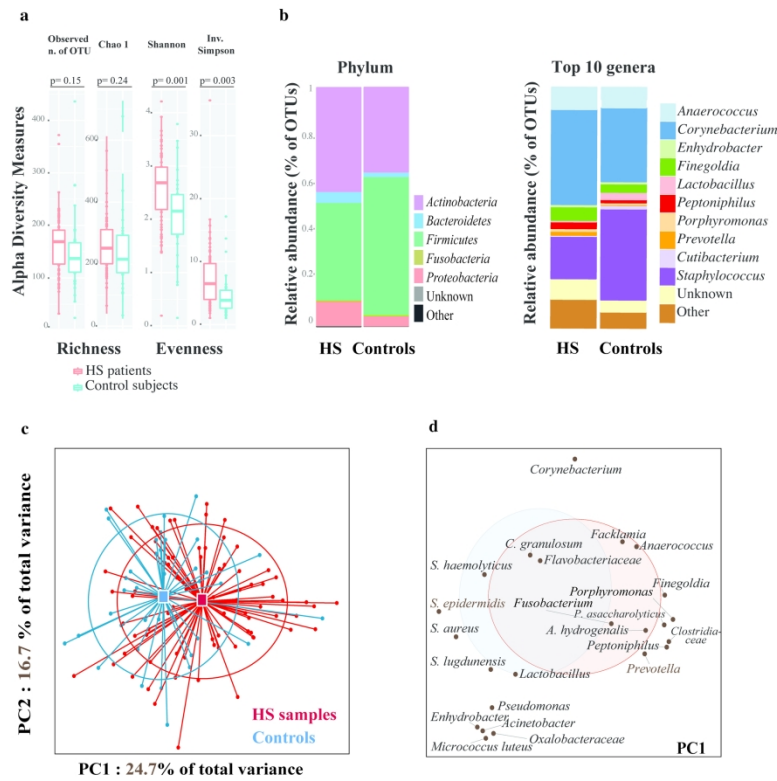


Fig1

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Figure 2

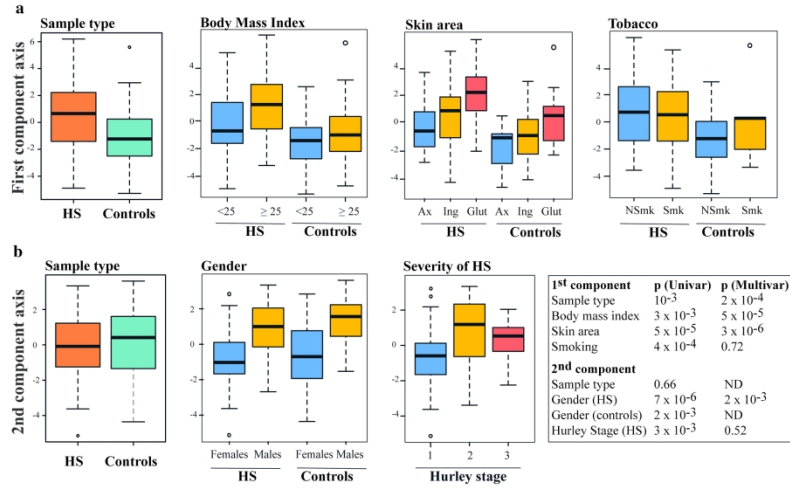


Figure 2

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Figure 3

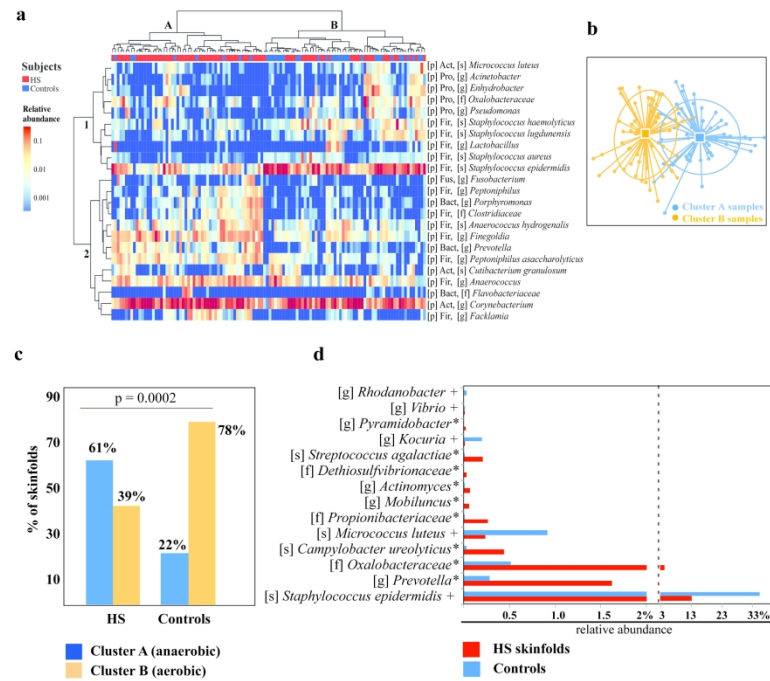


Figure 3

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**Table S1. Microbiology of clinically unaffected skinfolds in HS patients according to the clinical severity of the disease as assessed by bacterial cultures**

Bacterial species	Controls' samples (n=50)		Hurley St. 1 samples (n=87)		Hurley St. 2 & 3 samples (n= 55)		P*	FDR*
	F	mA	F	mA	F	mA		
<b><i>Corynebacterium spp</i></b>	<b>38</b>	<b>135</b>	<b>38</b>	<b>268</b>	<b>75</b>	<b>676</b>	<b>2. 10<sup>-5</sup></b>	<b>4. 10<sup>-4</sup></b>
<i>C. tuberculostearicum</i>	40	214	40	193	27	134	0.15	0.35
<i>Cutibacterium acnes</i>	12	49	2	12	0	0	0.26	0.53
<i>Cutibacterium avidum</i>	36	205	28	58	20	57	0.38	0.63
<b><i>Cutibacterium spp</i></b>	<b>4</b>	<b>10</b>	<b>13</b>	<b>15</b>	<b>0</b>	<b>0</b>	<b>0.01</b>	<b>0.05</b>
<i>S. aureus</i>	2	2	6	36	13	41	0.16	0.35
<i>S. epidermidis</i>	82	518	74	352	64	306	0.42	0.65
<i>S. hominis</i>	42	178	22	99	22	103	1.00	1.00
<i>S. haemolyticus</i>	18	104	25	154	15	67	0.12	0.35
<i>S. lugdunensis</i>	12	30	26	82	22	81	0.64	0.85
Other CoNS	34	156	18	73	9	37	0.13	0.35
Micrococcaceae	30	16	16	16	16	68	0.80	0.94
<i>Dermabacter hominis</i>	48	214	37	177	56	223	0.06	0.23
Enterobacteriaceae	18	43	32	155	27	125	0.61	0.85
<i>Enterococcus spp</i>	18	76	33	135	16	77	0.03	0.16
<b><i>Actinomyces neuii</i></b>	<b>14</b>	<b>66</b>	<b>22</b>	<b>109</b>	<b>5</b>	<b>6</b>	<b>0.01</b>	<b>0.05</b>
<i>Actinomyces spp</i>	0	0	2	12	2	0	0.84	0.94
Milleri group streptococci	0	0	3	17	4	19	0.96	1.00
<i>Streptococcus agalactiae</i>	4	21	5	46	9	62	0.31	0.56
Anaerobes	32	21	52	259	55	295	0.85	0.94

F: frequency (% of positive samples). mA= mean abundance (n° of colonies/sample).

CoNS: coagulase negative staphylococci. Taxa in bold: significant mean abundance difference between the two samples groups.

\* Statistical analysis: Hurley stage 1 - vs Hurley stage 2 and 3 patients' samples.

**Table S2. Bacterial taxa differentially associated with gender (genus and species level, FDR ≤ 0.05)**

HS patients	Females		Males		P	FDR
	F	mAb	F (%)	mAB		
[Fir] [s] <i>Lactobacillus iners</i> *	0.24	0.61	0	0	3.8 10 <sup>-5</sup>	0.005
[Fir] <i>Lactobacillus</i> *	0.24	0.19	0	0	5.0 10 <sup>-5</sup>	0.005
[Act] [s] <i>Kocuria rhizophila</i> *	0.53	0.37	0.32	2.7 10 <sup>-2</sup>	9.7 10 <sup>-5</sup>	0.007
[Pro] [g] <i>Ralstonia</i> *	0.38	1.3	0.12	5.1 10 <sup>-3</sup>	2.1 10 <sup>-4</sup>	0.013
[Pro] [g] <i>Trabulsiella</i> *	0.27	0.51	0.06	1.3 10 <sup>-3</sup>	2.5 10 <sup>-4</sup>	0.013
[Pro] [s] <i>Sphingomonas yabuuchiae</i> *	0.27	2.3 10 <sup>-2</sup>	0.03	6.4 10 <sup>-4</sup>	4.1 10 <sup>-4</sup>	0.018
[Pro] [g] <i>Paracoccus</i> *	0.42	0.31	0.24	1.5 10 <sup>-2</sup>	4.7 10 <sup>-4</sup>	0.018
[Fir] [s] <i>Staphylococcus lugdunensis</i> *	0.76	1.3	0.47	0.19	5.3 10 <sup>-4</sup>	0.018
[Pro] [g] <i>Neisseria</i> *	0.20	6.2 10 <sup>-2</sup>	0.03	6.4 10 <sup>-4</sup>	1.1 10 <sup>-3</sup>	0.032
[Fir] [s] <i>Veillonella dispar</i> *	0.18	1.6 10 <sup>-2</sup>	0	0	1.3 10 <sup>-3</sup>	0.035
[Bact] [g] <i>Chryseobacterium</i> *	0.18	6.9 10 <sup>-2</sup>	0.03	6.4 10 <sup>-4</sup>	1.5 10 <sup>-3</sup>	0.036
[Act] [s] <i>Brevibacterium paucivorans</i> +	0.13	6.9 10 <sup>-3</sup>	0.44	0.69	5.2 10 <sup>-7</sup>	2.0 10 <sup>-4</sup>
[Bact] [g] <i>Kaistella</i> +	0	0	0.12	8.5 10 <sup>-2</sup>	1.4 10 <sup>-3</sup>	0.036

Controls	Females		Males		p	FDR
	F	mAb	F	mAB		
[Act] [s] <i>Micrococcus luteus</i> *	0.82	1.57	0.60	6.9 10 <sup>-2</sup>	2.0 10 <sup>-4</sup>	0.013
[Fir] [g] <i>Lactobacillus</i> *	0.45	5.2 10 <sup>-2</sup>	0	0	2.2 10 <sup>-4</sup>	0.013
[Fir] [s] <i>Lactobacillus crispatus</i> *	0.41	1.67	0	0	8.8 10 <sup>-5</sup>	0.013
[Fir] [s] <i>Lactobacillus iners</i> *	0.45	3.63	0	0	4.0 10 <sup>-5</sup>	0.012
[Fir] [s] <i>Staphylococcus lugdunensis</i> *	0.86	1.9	0.60	0.16	1.9 10 <sup>-4</sup>	0.013
[Fir] [g] <i>Parvimonas</i> +	0.18	4.1 10 <sup>-3</sup>	0.33	1.8	4.4 10 <sup>-4</sup>	0.022

F: frequency (% of positive samples), mA= mean relative abundance (%)

\*: taxa associated with females' skinfolds. +: taxa associated with males' skinfolds.

FDR: false discovery rate.

[Bact]: phylum *Bacteroidetes*, [Fir]: phylum *Firmicutes*, [Act]: phylum *Actinobacteria*, [Pro]: phylum *Proteobacteria*. [g]: genus, [s]: species

**Table S3. Bacterial taxa significantly associated with clusters A (anaerobic) and B (aerobic) samples as identified by hierarchical clustering analysis.**

Cluster	Phylum	Taxa	Cluster A		Cluster B		FDR
			F	mA	F	mA	
Cluster A (anaerobic)	Actinobacteria	[s] <i>Actinomyces europaeus</i>	37.5	0.10	1.67	0.0003	5.9 10 <sup>-09</sup>
	Actinobacteria	[s] <i>Pseudoclavibacter bifida</i>	21.4	0.02	8.33	0.002	0.03
	Actinobacteria	[g] <i>Mobiluncus</i>	39.3	0.07	6.7	0.006	0.007
	Actinobacteria	[s] <i>Varibaculum cambriense</i>	50.0	0.16	16.7	0.010	0.0001
	Actinobacteria	[f] <i>Propionibacteriaceae</i>	51.8	0.35	25.0	0.020	0.0001
	Actinobacteria	[g] <i>Actinomyces</i>	58.9	0.10	30.0	0.028	0.03
	Actinobacteria	[s] <i>Rothia mucilaginosa</i>	50.0	0.47	35.0	0.040	0.0002
	Bacteroidetes	[o] <i>Bacteroidales</i> [f] S24-7	16.1	0.02	0	0	0.0004
	Bacteroidetes	[o] <i>Bacteroidales</i>	14.3	0.005	0	0	0.005
	Bacteroidetes	[g] <i>Kaistella</i>	7.14	0.05	0	0	0.023
	Bacteroidetes	[s] <i>Bacteroides cacae</i>	5.36	0.28	0	0	0.04
	Bacteroidetes	[s] <i>Bacteroides fragilis</i>	12.5	0.55	3.33	0.0007	0.007
	Bacteroidetes	[s] <i>Bacteroides plebeius</i>	32.1	0.19	10.0	0.014	0.04
	Bacteroidetes	[f] <i>Flavobacteriaceae</i>	10.7	1.60	16.7	0.017	0.02
	<b>Bacteroidetes</b>	<b>[g] <i>Porphyromonas</i></b>	<b>83.9</b>	<b>1.94</b>	<b>41.7</b>	<b>0.058</b>	<b>7.7 10<sup>-12</sup></b>
	<b>Bacteroidetes</b>	<b>[g] <i>Prevotella</i></b>	<b>89.3</b>	<b>2.29</b>	<b>46.7</b>	<b>0.129</b>	<b>7.7 10<sup>-11</sup></b>
	Firmicutes	[s] <i>Moryella indoligenes</i>	16.1	0.01	0	0	0.0005
	Firmicutes	[s] <i>Megasphaera geminatus</i>	10.7	0.01	0	0	0.007
	Firmicutes	[g] <i>Helcococcus</i>	8.93	0.03	0	0	0.01
	Firmicutes	[s] <i>Eggerthella lenta</i>	8.93	0.02	0	0	0.02
	Firmicutes	[f] <i>Coprobaclaceae</i>	8.93	0.01	0	0	0.02
	Firmicutes	[s] <i>Eubacterium dolichum</i>	7.14	0.006	0	0	0.04
	Firmicutes	[s] <i>Dialister micraerophilus</i>	37.5	0.07	3.33	0.003	3.0 10 <sup>-5</sup>
	Firmicutes	[g] <i>Atopobium</i>	19.6	0.04	6.67	0.003	0.02
	Firmicutes	[g] <i>Peptococcus</i>	30.4	0.05	10.0	0.005	0.01
	Firmicutes	[f] <i>Veillonellaceae</i>	42.9	0.10	13.3	0.005	10 <sup>-5</sup>
	Firmicutes	[g] <i>Parvimonas</i>	41.1	0.61	25.0	0.008	1.2 10 <sup>-06</sup>
	Firmicutes	[g] <i>Clostridium</i>	46.4	0.07	20.0	0.013	0.01
	<b>Firmicutes</b>	<b>[g] <i>Dialister</i></b>	<b>69.6</b>	<b>0.34</b>	<b>26.7</b>	<b>0.014</b>	<b>1.1 10<sup>-11</sup></b>
	Firmicutes	[g] <i>Negativicoccus</i>	58.9	0.17	33.3	0.025	0.0001
	Firmicutes	[g] <i>Facklamia</i>	62.5	2.10	33.3	0.064	7.1 10 <sup>-07</sup>
	<b>Firmicutes</b>	<b>[f] <i>Clostridiaceae</i></b>	<b>78.6</b>	<b>1.43</b>	<b>48.3</b>	<b>0.0768</b>	<b>1.6 10<sup>-10</sup></b>
	Firmicutes	[g] <i>Peptoniphilus</i>	76.8	0.90	36.7	0.079	1.8 10 <sup>-07</sup>
	<b>Firmicutes</b>	<b>[s] <i>P. asaccharolyticus</i></b>	<b>100</b>	<b>3.22</b>	<b>78.3</b>	<b>0.488</b>	<b>8.1 10<sup>-11</sup></b>
	Firmicutes	[s] <i>A. hydrogenalis</i>	96.4	3.36	81.7	1.139	0.022
	Firmicutes	[g] <i>Finegoldia</i>	98.2	8.53	88.3	1.431	5.3 10 <sup>-10</sup>
	Fusobacteria	[g] <i>Fusobacterium</i>	48.2	0.84	25.0	0.0340	0.0002
	Proteobacteria	[s] <i>Oligella urethralis</i>	8.93	0.31	0	0	0.008
	Proteobacteria	[g] <i>Campylobacter</i>	35.7	0.052	8.33	0.0037	0.0002
	Proteobacteria	[s] <i>C. ureolyticus</i>	78.6	0.594	23.3	0.0256	1.9 10 <sup>-09</sup>
	Synergistetes	[f] <i>Dethiosulfovibrionaceae</i>					
Synergistetes	[g] <i>Pyramidobacter</i>	17.9	0.028	1.67	0.0004	0.002	
Synergistetes	[f] <i>Dethiosulfovibrionaceae</i>	26.8	0.034	1.67	0.0007	7.0 10 <sup>-5</sup>	

Cluster B (aerobic)	Actinobacteria	[s] <i>Kocuria palustris</i>	8.93	0.013	35.0	0.129	0.04
	Actinobacteria	[s] <i>Micrococcus luteus</i>	48.2	0.159	66.7	0.735	0.01
	Cyanobacteria	[o] <i>Streptophyta</i>	12.5	0.009	15.0	0.414	0.05
	Firmicutes	[s] <i>Staphylococcus equorum</i>	0	0	8.33	0.005	0.04
	<b>Firmicutes</b>	<b>[g] <i>Staphylococcus</i></b>	<b>46.4</b>	<b>0.03</b>	<b>91.7</b>	<b>0.192</b>	<b>6.1 10<sup>-11</sup></b>
	<b>Firmicutes</b>	<b>[s] <i>Staphylococcus aureus</i></b>	<b>46.4</b>	<b>0.04</b>	<b>90.0</b>	<b>1.136</b>	<b>3.1 10<sup>-12</sup></b>
	Firmicutes	[s] <i>S. lugdunensis</i>	57.1	0.18	76.7	1.6214	7.8 10 <sup>-06</sup>
	<b>Firmicutes</b>	<b>[s] <i>S. haemolyticus</i></b>	<b>67.9</b>	<b>0.31</b>	<b>96.7</b>	<b>4.151</b>	<b>1.6 10<sup>-10</sup></b>
	Firmicutes	[s] <i>S.epidermidis</i>	96.4	7.18	100	32.19	9.8 10 <sup>-07</sup>
	Proteobacteria	[f] <i>Xanthobacteraceae</i>	5.36	0.003	23.3	0.0296	0.04
	Proteobacteria	[g] <i>Comamonas</i>	0	0	6.67	0.0435	0.04
	Proteobacteria	[g] <i>Agrobacterium</i>	7.14	0.004	16.7	0.1097	0.04
	Proteobacteria	[f] <i>Aeromonadaceae</i>	10.7	0.01	21.7	0.2118	0.02
Proteobacteria	[g] <i>Trabulsiella</i>	10.7	0.005	30.0	0.5081	0.0001	

F: frequency (% of positive samples). mA= mean relative abundance (%).  
 [o]: order, [f]: family, [g]: genus, [s]: species. Taxa in bold: FDR < 10<sup>-10</sup>

**Table S4. Bacterial taxa differentially associated with the clinical severity of the disease (genus and species level, FDR  $\leq$  0.1)**

Bacteria taxa	Hurley Stage 1 patients' samples		Stage 2 and 3 patients' samples		P	FDR
	F	mAb	F	mAb		
[Fir] [s] <i>Staphylococcus aureus</i> *	0.72	0.14	0.51	1.48	8.6 10 <sup>-5</sup>	0.03
[Act] [g] <i>Corynebacterium</i> *	1.00	26.6	1.00	53.5	2.4.10 <sup>-3</sup>	0.09
[Act] [s] <i>Pseudoclavibacter bifida</i> *	0.08	0.002	0.23	0.024	2.8 10 <sup>-3</sup>	0.10
[Pro] [g] <i>Trabulsiella</i> +	0.28	0.56	0.08	0.003	6.2 10 <sup>-4</sup>	0.08
[Act] [s] <i>Actinomyces neuii</i> +	0.60	0.38	0.33	0.06	8.5 10 <sup>-4</sup>	0.08
[Fir] [g] <i>Staphylococcus</i> +	0.70	0.13	0.51	0.04	2.0 10 <sup>-3</sup>	0.09

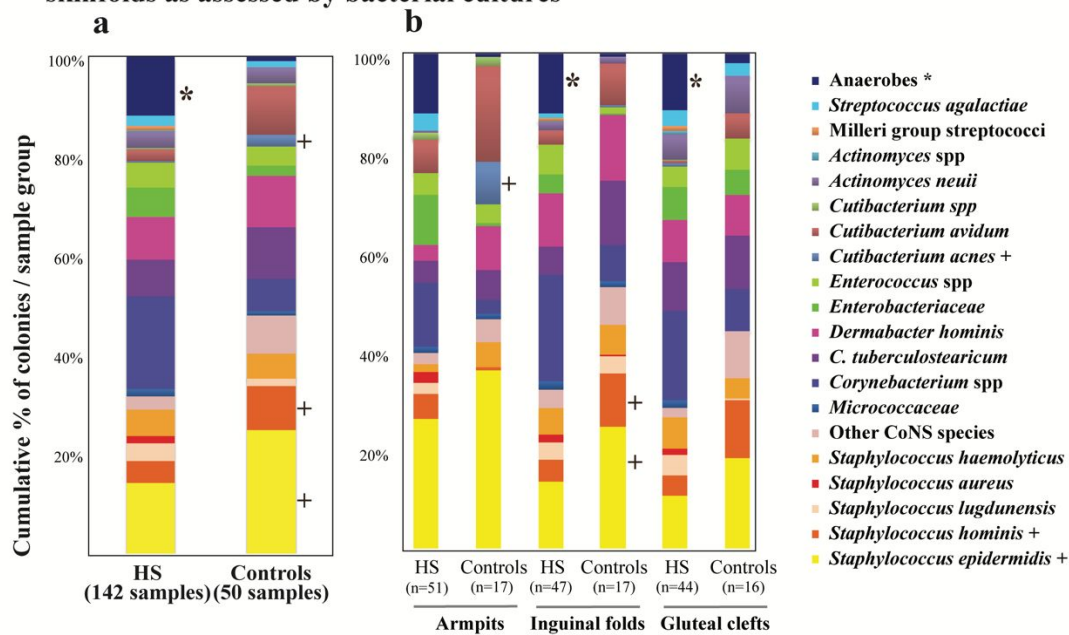
F: frequency (% of positive samples). mA= mean relative abundance (%).

\*: taxa associated with Hurley stage 2 and 3 patients' skinfolds. +: taxa associated with Hurley stage 1 patients' skinfolds. FDR: false discovery rate.

[Fir]: phylum Firmicutes, [Act]: phylum Actinobacteria, [Pro]: phylum Proteobacteria.

[g]: genus, [s]: species

**Figure S1. Predominant bacterial taxa of Hidradenitis Suppurativa clinically unaffected skinfolds as assessed by bacterial cultures**



a: all samples. b: microbiological profiles according to localization.

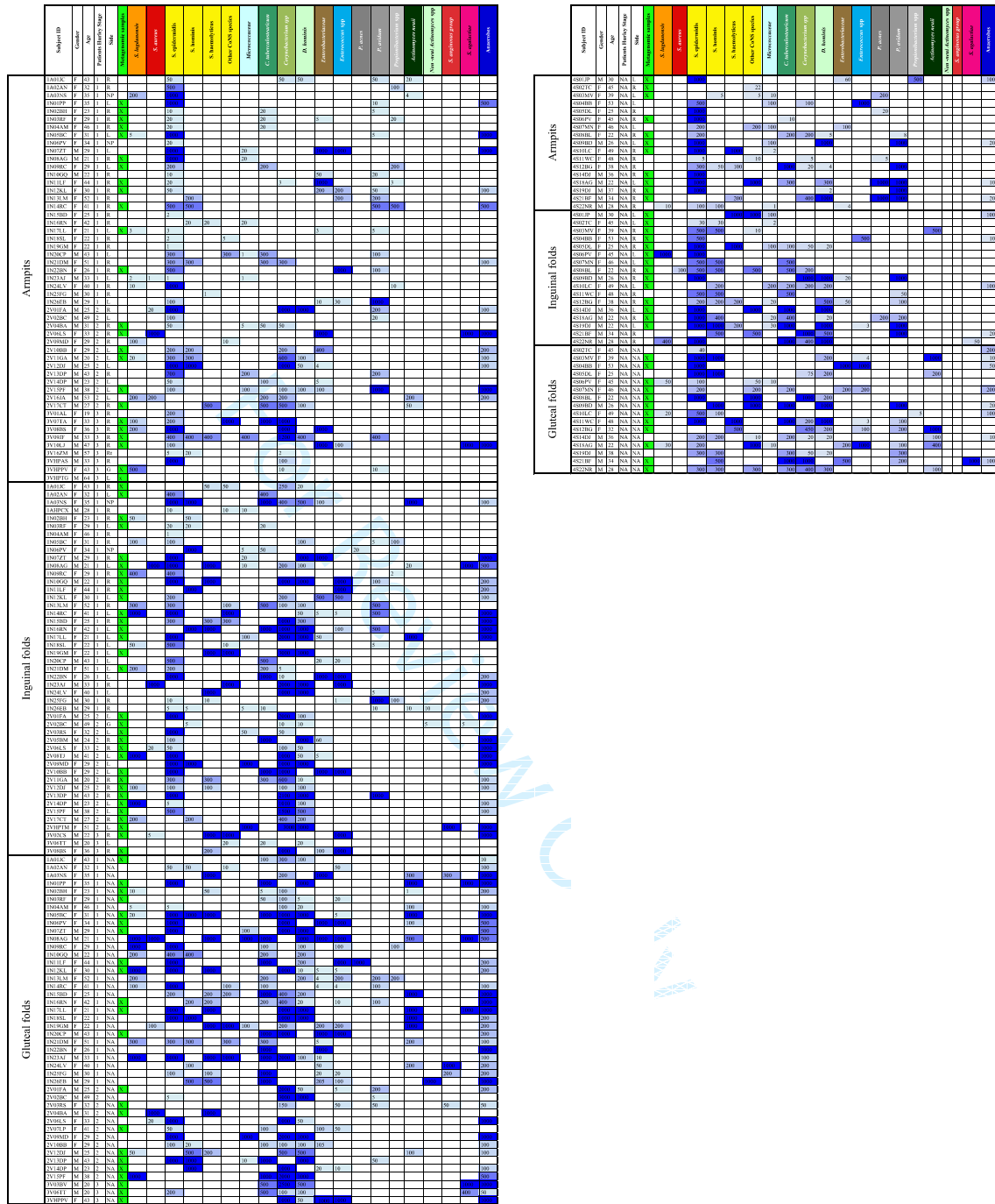
\*: bacterial taxa statistically associated with HS patients' unaffected skinfolds.

+ : bacterial taxa statistically associated with control subjects' skinfolds.

Figure S2. Individual bacterial culture results and samples submitted to 16S ribosomal RNA gene amplicon sequencing.

HS patients

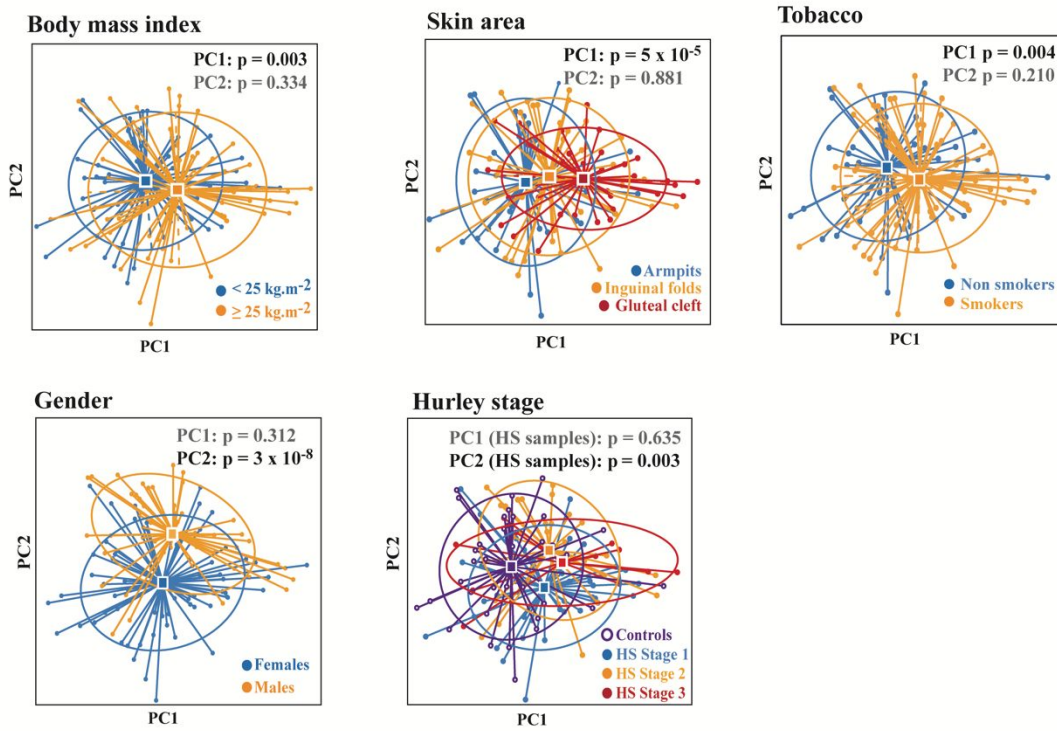
Controls



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Figure S3. Representation of clinical factors associated with microbiomes on the PCA ordination plot



PC1  $p$  = result of univariate analysis on the first component axis

PC2  $p$  = result of univariate analysis on the second component axis. For Hurley stage, we did not include controls' microbiomes.