The aim of this study was to identify key microbes associated with change in skin status (lesional vs normal). Longitudinal changes in the skin microbiome between patients with psoriasis and healthy family controls living in the same household were studied using whole genome metagenomic shotgun sequencing at 4 time-points. There were significant changes in abundance of the pathogen *Campylobacter jejuni* and its higher taxonomic levels when the skin status of patients with psoriasis changed. There were significant longitudinal variations in alpha diversity (*p* < 0.001) and beta diversity (*p* < 0.05) of the skin microbiome in patients with psoriasis, but not in the healthy control group, which indicated composition of skin microbiome in patients with psoriasis was different from healthy control and was dynamically less stable. This study will serve as the basis for future temporal studies of the skin microbiome and probiotic therapeutics.

Key words: skin microbiome; psoriasis; skin status; microbial diversity.

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Skin, the largest organ of the human body, acts as a protective barrier against external pathogenic organisms and harmful substances (1). It also harbours trillions of bacteria, fungi, viruses, and archaea, which collectively constitute the skin microbiome (2). The skin microbiome interacts with human health and has the ability to fine-tune the immune system of the human host (3) by modulating the local inflammatory milieu (4) and controlling the expression level of factors related to the immune system, such as antimicrobial peptides (5).

Advances in next generation sequencing (NGS) techniques have stimulated research into the composition of the skin microbiome and its implications for skin health and disorders. A commonly used technique used to study the composition of the bacterial community in the skin microbiome is 16S RNA gene sequencing. Another recent technique, whole genome metagenomic shotgun sequencing (WGS), enables the classification of various microbial communities in addition to bacteria, and the investigation of the full complement of genomics, including its functional potential (6). The use of fast and efficient NGS techniques has revealed associations between the skin microbiome and the host cutaneous condition in a variety of skin diseases, including acne vulgaris (7, 8), atopic dermatitis (9, 10), rosacea (11), and psoriasis (12–16).

Psoriasis is a chronic, immune-mediated skin disease that affects approximately 2% of the population worldwide (17). It is a multifaceted disease, the development of which involves various genetic and environmental risk factors. The skin microbiome has also been shown to play a role in psoriasis. For example, alpha diversity, which measures the richness and evenness of the microbial community, is lower in psoriatic lesional skin compared with control skin (12, 13). An increased ratio of 2 bacterial phyla, *Firmicutes* to *Actinobacteria*, has been found in psoriatic lesional skin compared with normal skin (14). A toxic substance produced by some strains of *Staphylococcus aureus* has been shown to correlate with psoriasis severity (15). A longitudinal study has observed a larger pretreatment heterogeneity and an increased variation in the skin microbiota in psoriatic lesional skin compared with that of normal skin during treatment with ustekinumab (16). Despite these studies reporting differences in the skin microbiome between psoriatic lesional skin and control skin at a single time-point, or a temporal trend during treatment, it is unclear how the skin microbiome changes with skin status. Few studies have examined whether the skin microbiome is associated with the recovery and recurrence of skin lesions over time.

The aim of the current study was therefore to investigate the key skin microbes connected with change in skin status in psoriasis. A WGS technique was used to deter-
mine the temporal skin microbiome profile of patients and healthy family controls living in the same household. In addition, differences in longitudinal changes in the skin microbiome between patients with psoriasis and family controls were characterized.

**MATERIALS AND METHODS**

**Study design**

The study was designed to investigate the association between longitudinal changes in skin microbiome and skin status of patients with psoriasis. A household-based sampling design was used. Skin samples were collected at 4 time-points from patients and healthy family controls living in the same household: baseline, and 3 follow-up time-points (duration between 2 consecutive time-points, median (interquartile range; IQR) 5.8 months (3.6–8.3 months). The sampling time-points were related to the status of the skin lesions. In particular, the focus was on samples that related to changes in skin status, in order to determine the changes in the skin microbiome with flare-up or clearing of the lesions.

This study was approved by the Institutional Review Board of the University of Hong Kong/Hospital Authority Hong Kong West Cluster (project UW-085). Written informed consent was obtained from all study participants before the study began. Eligible participants with psoriasis were recruited from the Family Cohort (18) and the Hong Kong Psoriasis Patients Association with baseline skin samples (19), with subsequent skin status changes at follow-up time-points.

**Subjects**

A total of 12 subjects were recruited from 6 different households. Subjects (patients and family controls) from 5 households underwent collection of skin samples at 4 time-points, and subjects from 1 household at 3 time-points. Subjects’ ages ranged from 33 to 75 years at the time of recruitment. Eligible family controls were subjects with healthy skin condition who lived in the same household as a patient. Subjects who had used antibiotic treatment (systemic or topical) within one month prior to sample collection at baseline were excluded. Five of the 6 patients had taken topical steroids within 30 days before samples were obtained during at least one of their visits. Only one of the 6 patients had received biologic therapy within 30 days before samples were obtained during at least one of their visits. None of the patients reported loss to follow-up.

**Skin sample collection**

All subjects agreed to provide skin swab specimens. Eligible cases were psoriasis patients with a lesion and a contralateral non-lesion in at least one of the following locations: elbow, forearm, knee, or scalp at baseline, and a change in skin status in at least one of these locations at follow-up. Samples from the left side and right side of different body locations of eligible cases were also collected. Samples from one side of the matched body locations of family controls were collected. ESwarb (Copan Diagnostics, Murrieta, CA, USA) was used to collect skin swab specimens from all subjects. Each skin swab sample was taken from a circle with a radius of approximately 2 cm by swabbing back and forth 25 times. Genomic DNA from each skin sample was extracted using a QIAamp DNA Mini Kit (QIAGEN, Germantown, MD, USA), following the manufacturer’s instructions for swab specimens. The DNA library was prepared using the KAPA HyperPrep Kit (Kapa Biosystems, Wilmington, MA, USA). Shotgun sequencing was performed using a HiSeq 1500 platform (Illumina, San Diego, CA, USA, 2 ×100 bp paired-end sequencing) for baseline samples and NovaSeq 6000 system (Illumina, San Diego, CA, USA, 2 ×150 bp paired-end sequencing) for the follow-up samples.

**Microbial operational taxonomic units annotation**

Raw sequence reads aligned to adapters and human contaminant were removed for the quality control. Remaining reads were assembled into contigs (long continuous sequences) by IDBA-UD (20). Classification, clustering and annotation of the microbial operational taxonomic units (OTUs) were performed using DIAMOND (21) and MEGAN (22). MEGAN long read (23) mode was used for the binning of OTUs, as contigs were the input data. Relative abundance (%) of normalized read count of annotated OTUs from different taxonomic ranks by MEGAN was used as the estimation of microbial abundance.

**Data analysis and statistics**

R statistical software (24) was used for all statistical analyses and data visualization. Orders of the abundance of OTUs were ranked based on the median of the mean relative abundance of the skin samples from the same subject and body location over time. Low abundant OTUs (relative abundance <0.1%) were filtered out. Alpha and beta diversities were determined as described previously (19). In order to determine the key taxa associated with the change in skin status, a reference distribution of the change in the relative abundance between 2 time-points was calculated from control skin samples. Four types of change in skin status: lesion to normal; normal to lesion; lesion to lesion; and normal to normal were defined for patients’ skin samples. Distributions of the change in relative abundance in an investigated microbe were compared between patients and controls among 4 different types of change in skin status using a permutation test. Longitudinal patterns of microbial relative abundance levels over different time-points were quantified by the first principal component (PCA1) of principal component analysis (PCA). A linear mixed effects model using lme4 (25) was applied to compare differences in longitudinal changes in microbial abundance, microbiome diversities, and to provide estimates of the longitudinal pattern between patients and controls, adjusting for within-subjects effect and sex. False-positive rate (FDR) was used to adjust for the multiple comparisons. R packages ggplot2 (26) and ggtree (27) were used to visualize the results.

**RESULTS**

**Summary of study subjects and samples obtained**

A total of 141 skin samples were collected from 12 subjects at 4 time-points: 24 samples from patients and 12 from family controls at baseline, first and second follow-ups; and 22 samples from patients and 11 from family controls at third follow-up (one household was lost to follow-up).

**Composition of the skin microbiota**

Fig. S1 summarizes the composition of the top 10 most abundant taxa at different taxonomic levels in patients’ lesional skin, patients’ normal skin, and controls’ normal
skin over different time-points. At the phylum level (Fig. S1a), Proteobacteria (median (IQR) 52.5% (39.6–64.0%)), Actinobacteria (31.2% (20.7–46.1%)), Bacteroidetes (8.5% (2.9–15.4%)), and Firmicutes (1.4% (0.6–3.1%) were identified. These 4 major phyla have also been reported in other skin microbiome-related studies (1, 28, 29). The median relative abundances of other phyla were less than 1%. At the family level (Fig. S1b), the 3 most abundant families were Pseudomonadaceae (18.4% (1.2–31.2%)), Propionibacteriaceae (11.4% (3.9–21.3%)) and Moraxellaceae (6.2% (2.7–12.4%)). Pseudomonadaceae and Moraxellaceae are both in the phylum Proteobacteria. At the genus level, Pseudomonas was the most dominant genus (22.2 (1.5–37.2%)), and the dominance of Pseudomonas was documented previously (30). Acinetobacter, Moraxella and Propionibacterium were other abundant genera that had a median relative abundance >2%. At the species level, a total of 1,364 different species were annotated. The top 10 most abundant species accounted for 12.8% of the total bacterial community, according to the median of the mean relative abundance over time.

**Key taxa and change in skin status**

To investigate whether a microbe was associated with the change of skin status in patients vs controls, permutation test with 999 permutations was used to compare the change in relative abundance in a microbe among 4 different types of change in skin status between patients and controls. 4 microbial taxa had one or more types of change with significant differences. Fig. S2 shows box-plot comparison of the extent of changes in relative abundance in different types of change in skin status and the reference for these taxa. The significantly different comparisons were indicated by an asterisk. In particular, the species Campylobacter jejuni and its higher taxonomic genus, the Campylobacter bacter, and the Campylobacteraceae family, all showed significant differences between the transition from lesion to normal status and the reference normal with respect to the distribution of change in relative abundance (Fig. S2a–c). Compared with the reference distribution, all Campylobacter jejuni-related taxa underwent a significantly more negative change in relative abundance when the skin status changed from lesion to normal. Conversely, for the transition from normal to lesion, these taxa consistently underwent a more positive change in relative abundance compared with the reference, and a significance change was observed in the Campylobacteraceae family. The Gordoniaceae family showed a more positive change in its relative abundance for the transition from lesion to normal for patients vs controls. And conversely, a significantly more negative change of relative abundance in Gordoniaceae for the transition from normal to lesion was observed. (Fig. S2d).

**Longitudinal changes in major taxa and microbial diversity**

Fig. S3a shows the phylogenetic relationship between taxa identified as showing significant longitudinal changes (adjusted \( p < 0.05 \), linear mixed effects model) at different taxonomic levels and in different groups of skin samples. Most of the taxa with significant longitudinal changes were in the phylum Proteobacteria and from skin samples from patients. The genus Acinetobacter and its lower level species, Acinetobacter junii and Acinetobacter baumannii; family Sphingomonadaceae and its lower level genera, Sphingomonas and Sphingobium; and family Caulobacteraceae changed over time in skin samples from patients. Phylum Chloroflexi changed over time in skin samples from family controls. The family Comamonadaceae changed over time in skin samples from both patients and controls. Microbial alpha and beta diversities were calculated from the abundance profile at the species level to evaluate longitudinal changes in skin microbiota in patients and family controls. The alpha diversity measured using the Shannon diversity index revealed a significant change over time in patients with psoriasis (\( p < 0.001 \), linear mixed effects model), while there were no significant changes over time in the control group (\( p = 0.4 \)) (Fig. S3b). In addition, levels of alpha diversity in patient skin were lower than in control skin at each time-point. Beta diversity, estimated using PCoA1, changed significantly over time in skin samples from patients (\( p < 0.05 \)), but not in skin samples from family controls (\( p = 0.1 \))(Fig. S3b).

**Longitudinal pattern in relative abundance of taxa between patients and controls**

Although no significance was found with the adjustment for multiple comparisons, 4 taxa, Corynebacterium and Mucilaginibacter genera, Tsukamurella tyrosinosolvens and Janibacter indicus species, had raw \( p \)-values < 0.05. The mean relative abundances and the standard error of the mean over different time-points for these 4 taxa in skin samples from patients and controls are shown in Fig. S4. Four taxa were not significantly differential in the longitudinal pattern between patients and controls after the adjustment. However, at 2 or more time-points, the range of relative abundances of these taxa between patients and controls, measured as the mean and standard error of the mean, were visibly separated.

**DISCUSSION**

The skin microbiome can modulate the host immune system and is associated with the pathogenesis of a number of skin disorders. Advances in sequencing techniques of-
...fer more reliable, faster and more affordable approaches to analysing the composition of the skin microbiome. Recent research into the skin microbiome in diseased and healthy hosts has investigated its temporal stability (31, 32) and temporal variations (16, 28, 33) after receiving skin therapy. Psoriasis is a chronic skin disorder, in which the patient’s skin status can switch between lesion and normal over time. However, there is little published research regarding the microbes correlated with this change in skin status. The current study investigated longitudinal changes in the skin microbiome in patients with psoriasis and in healthy family controls over a period of one year. The most important finding of this study was the identification of key microbial taxa associated with the change in skin status in people with psoriasis. The relative abundance of *Campylobacter jejuni* species and its higher taxonomic levels, *Campylobacter* genus, and *Campylobacteraceae* family, were found to be significantly reduced when the skin status changed from lesional to normal, but increased when the skin status changed from normal to lesional. *Campylobacter jejuni* and its related microbial taxa were not statistically significant in the longitudinal analyses. This implies that the change in relative abundance of these microbes pooled across pairwise time-points were associated with the change in skin status, but were not present when longitudinal dynamics and patterns were considered. *Campylobacter jejuni*, is a leading pathogen causing bacterial gastroenteritis, including a potentially fatal paralytic autoimmune disorder as secondary sequelae (34). In addition, *Campylobacter jejuni* is associated with erysipelas-like skin lesions in patients with hypogammaglobulinaemia (35). A strong association between anti-*Campylobacter fetus* antibodies and psoriatic arthritis has been observed (36). Skin infection with *Campylobacter bacteremia* is a frequent manifestation in immunocompromised patients (37). Furthermore, *Campylobacter* infections are associated with a variety of autoimmune disorders, such as reactive arthritis, systemic lupus erythematosus and vasculitis (38). Our finding of an increase in Campylobacter during the change from normal to lesional skin in psoriasis, and a reduction in *Campylobacter jejuni* during the change from lesional to normal skin suggest a link between *Campylobacter jejuni* and the pathogenesis of psoriatic lesion. In addition, in the current study the *Gordoniaceae* family was significantly decreased when the skin status changed from normal to lesional, and increased when the skin status changed from lesional to normal. *Gordoniaceae* is in the phylum *Actinobacteria*. Although few studies have reported on the *Gordoniaceae* family, it has been observed previously that the reduction in phylum level *Actinobacteria* in psoriatic lesional skin is greater than that in the normal skin of both patients and controls (14). Our findings provide a detailed picture of the disease, by narrowing down the *Actinobacteria* phylum to its lower level family *Gordoniiaceae*, which may be negatively associated with psoriatic skin lesions.

In the current study, the longitudinal changes in the composition of the skin microbiome in patients with psoriasis and their healthy family controls was also delineated. The results regarding the 4 major phyla, *Proteobacteria, Actinobacteria, Bacteroidetes* and *Firmicutes*, in the skin microbiome, both in patients with psoriasis and in controls, were consistent with previous observations (1, 28, 29). The dominance of the genus *Pseudomonas* in the skin microbiome in the current study has also been reported previously (30). Moreover, we found significant longitudinal variations in alpha diversity, beta diversity and several microbial taxa in patients with psoriasis, but not in the healthy skin of family controls. The stability of the skin microbiome in healthy skin over time has been reported previously (32, 39). In addition, we found the alpha diversity at each time-point was lower in patients’ skin than in controls, which was consistent with the findings of previous cross-sectional studies (12, 13). The results of the current study imply a less stable composition of the skin microbiome in patients with psoriasis compared with healthy controls. These results also reveal the important role of a stable skin microbiome in the maintenance of healthy skin homeostasis.

In summary, using a novel household-based design, this study investigated the longitudinal dynamics of the composition of the skin microbiome in patients with psoriasis and healthy family controls at 4 time-points. Using healthy family controls as a reference, key microbial taxa associated with the change in skin status in patients with psoriasis were identified. Although numerous therapies have been licensed for psoriasis, the natural history of psoriasis remains unclear, and accessing appropriate therapy remains a challenge (40). With advances in understanding of the role of the skin microbiome in maintaining healthy skin, recent studies have examined the use of probiotic therapy to modulate the condition of the skin (41, 42). The results of the current study, in particular the identification of 2 key microbial taxa associated with changes in skin status, could indicate potential targets for probiotic therapy. Further research into the skin microbiome is needed to better understand the longitudinal dynamics of skin status in psoriasis and its implication for future therapeutics.

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