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ORIGINAL ARTICLE

Gut microbiota alterations in moderate to severe acne vulgaris patients

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ABSTRACT

Acne vulgaris is a chronic inflammatory dermatosis affecting approximately 85% of adolescents. There are many factors contributing to the development of this ailment. A recent study indicated that gut microbiota takes part in the pathogenesis of acne. We aimed to investigate the link between acne vulgaris and gut microbiota. A total of 31 moderate to severe acne vulgaris patients and 31 healthy controls were enrolled. We collected their feces, and gut microbiota was evaluated by the hypervariable regions of 16S rRNA genes through high-throughput sequencing. We identified links between acne vulgaris and changes of gut microbiota. At the phylum level, Actinobacteria (0.89% in acne patients and 2.84% in normal controls, $P = 0.004$) was decreased and Proteobacteria (8.35% in acne patients and 7.01% in normal controls, $P = 0.031$) was increased. At the genus level, *Bifidobacterium*, *Butyricicoccus*, *Coprobacillus*, *Lactobacillus* and *Allobaculum* were all decreased. The observed difference in genera between acne patients and healthy controls provides a new insight into the link between gut microbiota changes and acne vulgaris risk.

Key words: acne vulgaris, difference, gut microbiota, high-throughput sequencing, pathogenesis.

INTRODUCTION

Acne vulgaris is a common dermatological disorder affecting approximately 85% of adolescents. Moderate to severe acne occurs in approximately 20% of young people, and the severity correlates with pubertal maturity. Acne persists in the 20s and 30s in approximately 64% and 43% of individuals, respectively.[1] The cause of acne vulgaris remains to be determined. Ductal epidermal hyperproliferation, excessive sebum, inflammation and the presence of *Propionibacterium acnes* all contribute to the development of acne vulgaris,[2] but the extent to which these factors influence the attack of acne is unknown.

Previous studies suggest that gut microbiota may take part in the pathogenesis of acne. In 2001, Volkova *et al.*[3] studied 114 acne vulgaris patients (94 papulopustular form and 20 nodulocystic form) and found that 54% of the patients had either the first (21%) or second (78.7%) impaired intestinal microflora. One study involving over 6500 Chinese adolescents aged 12–20 years indicated that acne patients had higher prevalence of gastrointestinal symptoms (halitosis, gastric reflux, abdominal bloating and constipation) and that gastrointestinal dysfunction was an important risk factor for sebaceous gland diseases including acne and was correlated with their occurrence and development.[4] Recently, a study reported that patients with acne, papular–pustular rosacea and seborrheic dermatitis acquired significant amelioration or complete recovery and a shift toward a protective microbiota with predominance of *Bifidobacteria* and *Lactobacteria* was observed in most patients after oral application of the probiotic *Escherichia coli* Nissle 1917.[5]

To explore whether gut microbiota is related to the pathogenesis of acne vulgaris, we collected fecal samples from patients with moderate to severe acne vulgaris and matched healthy controls to examine the differences of gut microbiota between acne patients and healthy controls.

METHODS

Ethics statement

Written informed consent was obtained from each subject before collection of fecal samples. This study was approved by the institutional review board of Peking University Third Hospital.

SUBJECTS

We recruited 31 moderate to severe acne vulgaris patients (group P, 23 females and 8 males, 17–35 years old with an average age of 22.16 years) from our dermatology clinics between 10 October 2016 and 5 June 2017, and 31 age- and sex-matched normal controls without acne vulgaris and any other skin diseases (group N, 23 females and 8 males,

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Table 1. Information about acne patients (P) and healthy controls (N)

Index	Group N	Group P	*P*
Mean age, years	22.87 ± 3.65	22.16 ± 4.24	0.482
Sex (M/F)	8:23	8:23	1.000
BMI	21.76 ± 1.92	21.22 ± 1.69	0.242

BMI, body mass index.

19–31 years old with an average age of 22.87 years; Table 1). We also collected drinking and smoking habits of all subjects. Drinking habit was divided into: (i) no drinking; (ii) drinking about once a week; (iii) drinking about three times a week; and (iv) drinking every day. Smoking habit was divided into: (i) non-smoker; (ii) smoking but not every day; and (iii) smoking every day (Table 2). They had been living in Beijing for a long time. A complete dermatological examination was performed for each subject. Exclusion criteria included: having inflammatory bowel syndrome or any other systemic disease; and using systemic antibiotics, retinoids, corticosteroids or immunosuppressive agents within 2 months that seem to be sufficient for gut microorganism to recover from the changes by drugs.[6]

Sample collection and DNA extraction

Fecal samples were collected in sterilized containers and immediately stored at −20°C. On the day of fecal collection, fecal DNA was extracted using the E.Z.N.A. Feces DNA Kit (Omega Bio-tek, Norcross, GA, USA) and stored at −20°C. DNA was determined using the Qubit Fluorometer (Life Technologies, Carlsbad, CA, USA). DNA integrity and size were checked by 1% agarose gel electrophoresis.

The hypervariable V4 515-806 region of 16S rRNA in bacterial genomic DNA was first amplified using a set of forward primers containing the forward sequences of 5′-GTGYCAGCMGCCGCGGTAA-3′ around the site 515 and a set of reverse primers containing the reverse complement sequences of 5′-GGACTACNVGGGTWTCTAAT-3′ around the site 806.[7] The 62 fecal DNA samples were sequenced in an Illumina HiSeq platform according to manufacturer's instructions conducted by BGI Tech (BGI, Shenzhen, China).

Bioinformatic and statistical analysis

The high-quality paired-end reads from the original DNA fragments were combined to tags based on overlaps using FLASH,[8] which is designed to merge pairs of reads when original DNA fragments are shorter than twice the read length. Tags were clustered to operational taxonomic unit (OTU) at 97% sequence similarity by scripts of software USEARCH,[9] and the OTU unique representative sequences were obtained. Chimeras were filtered out from the OTU unique representative sequences by using UCHIME.[10] All tags were mapped to each OTU representative sequences using USEARCH GLOBAL,[11] and the tag number of each OTU in each sample was summarized in OTU abundance (Table S3). The OTU representative sequences were then taxonomically classified using Ribosomal Database Project (RDP) Classifier[12] trained on the Greengenes database,[13] using 0.8 confidence values as cut-off. The relative abundance values and sequence counts were all normalized.

Table 2. Drinking and smoking habits of acne patients (P) and healthy controls (N)

Lifestyle	Group N (%)	Group P (%)	*P*
Alcohol drinking habit			
No drinking	17 (54.8)	16 (51.6)	0.696
Once per week	11 (35.5)	14 (45.2)	
Three times per week	2 (6.5)	1 (3.2)	
Every day	1 (3.2)	0 (0)	
Smoking habit			
Non-smoker	28 (90.3)	30 (96.8)	0.612
Smoking, but not everyday	3 (9.7)	1 (3.2)	
Smoking everyday	0 (0)	0 (0)	

To evaluate the amount of diversity contained within communities (alpha diversity), the indices (Observed species value, Chao value) were calculated by Mothur (version 1.31.2; http://www.mothur.org/wiki/Calculators) and the corresponding boxplot was drawn by software R (v3.1.1). In order to display the differences of OTU composition in different samples, we also used principal component analysis (PCA) to summarize factors mainly responsible for this difference; similarity is high if two samples are closely located.

Statistical analyses were performed with SPSS version 20.0 (SPSS, Chicago, IL, USA) to determine the statistical differences between the two groups using parametric methods (Student's *t*-test), χ^2-test and non-parametric statistical methods (Mann–Whitney *U*-test). A value of $P < 0.05$ was considered to be statistically significant.

RESULTS

The high-quality paired-end reads were combined to tags based on overlaps; we obtained 8 343 289 tags from the 62 samples, and the mean number of tags was 134 569 (range, 81 328–163 039), which implies that tags from each sample had a few differences. A total of 4 080 836 tags were obtained from acne patients for phylogenetic analysis, while 4 262 453 tags were obtained from healthy controls. The chimeras and singletons were filtered out; we obtained 4 778 462 filtered tags, and they were then clustered into OTU with a 97% threshold by using UPARSE. A total of 1276 OTU were obtained from the 62 fecal samples (group P 1072 OTU and group N 1158 OTU; Fig. 1). To compare the microbiota composition of the two groups, a Venn diagram of OTU at a level of 97% similarity was generated (Fig. 1).

Microbiota diversity

The Good's coverage, which accounts for both diversity and abundance, was estimated to be 99.88% for both groups, indicating a sufficient sequencing depth for data comparison. This was also supported by the rarefaction curve of the 62 samples,

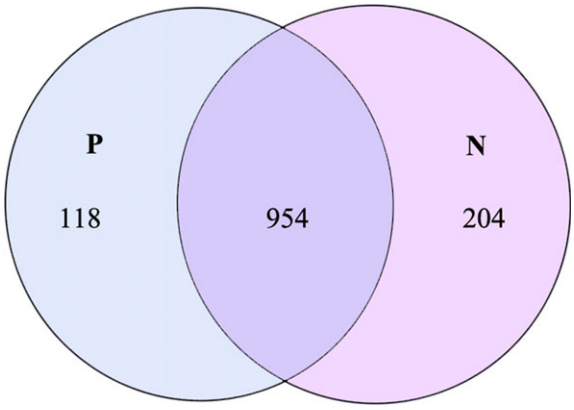

Figure 1. Venn diagram showing the unique and shared operational taxonomic unit in acne patient group (P) and healthy control group (N).

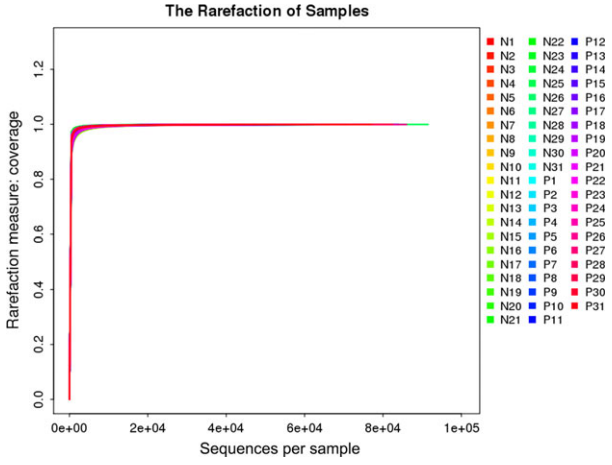

Figure 2. Coverage rarefaction curves; each curve tends to be smooth.

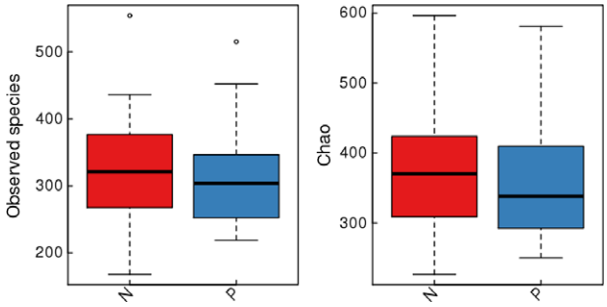

Figure 3. Alpha diversity comparison in group N versus group P (Observed species and Chao indexes).

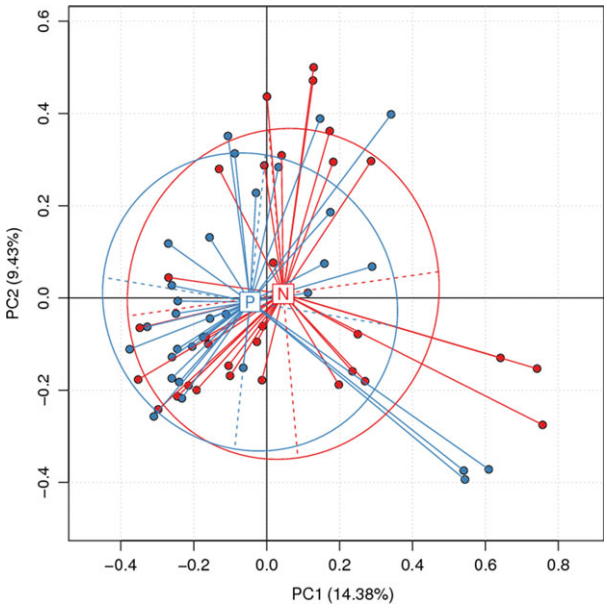

Figure 4. Principal component analysis. Each dot represents one sample. Blue colors represent group P (patients), and red colors represent group N (healthy controls). The two principal coordinates, PC1 and PC2, explain 14.38% and 9.43%, respectively.

which tends to be asymptotic suggesting that the most shared species have been obtained (Fig. 2). To determine the richness and the diversity involved, we calculated different diversity indices (Chao, Sobs) and found that, regardless of the used metric, the richness and diversity of the two groups had no significant differences (Fig. 3). In sum, although some special genera had statistically significant difference, the OTU differences were insufficient to separate them into two distinct clusters as revealed by principal component analysis (PCA; Fig. 4).

Distribution at phylum level

The 1276 OTU belong to 19 phyla. At the phylum level, Bacteroidetes, Firmicutes, Proteobacteria and Actinobacteria were the main four phyla that consisted of the gut microbiota of acne patients and healthy controls (Fig. 5, Table S1), consistent with the previous research results.[14,15] Bacteroidetes was the most abundant bacterial phylum (49.15% in group P and 44.50% in group N), followed by Firmicutes (40.71% in group P and 44.48% in group N), Proteobacteria (8.35% in group P and 7.01% in group N) and Actinobacteria (0.89% in group P and 2.84% in group N). The proportions of rare phyla including Verrucomicrobia, Synergistetes, Tenericutes, Lentisphaerae, Elusimicrobia, Chlorobi and Cyanobacteria were present at much lower levels. We found that Actinobacteria was much higher in group N than in group P (2.844% vs 0.895%, $P = 0.004$), while Proteobacteria was much lower in group N than in group P (7.014% vs 8.351%, $P = 0.031$; Table 3).

Distribution at genus level

At the genus level, the tags from fecal microbiota represented 144 bacterial genera, of which 134 genera were in acne patients, and 130 genera in healthy controls. *Bacteroides*, *Prevotella*, *Blautia*, *Clostridium*, *Coprococcus*, *Dialister*,

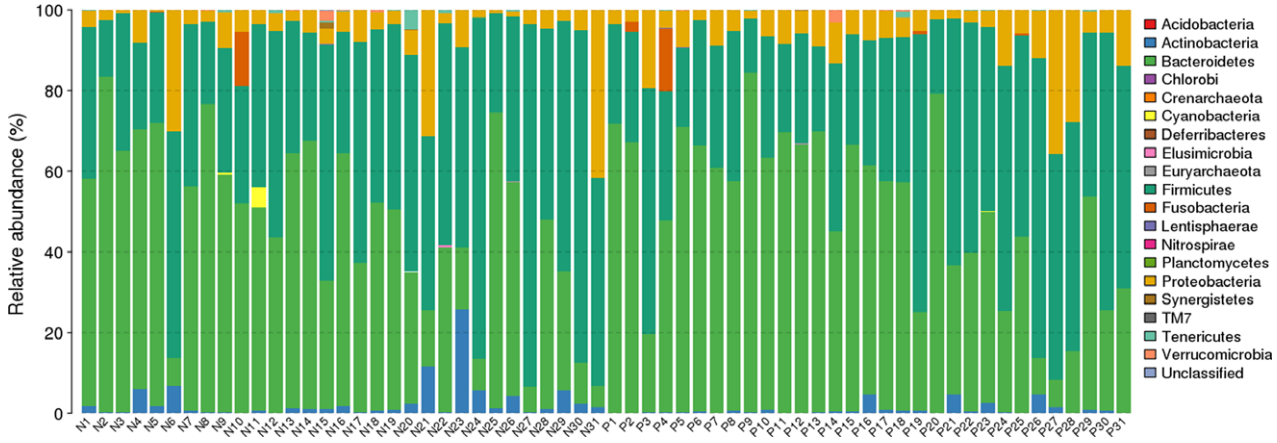

Figure 5. Relative abundance of different bacteria at phylum level in each sample.

Table 3. Major taxonomic differences between acne patients (P) and healthy controls (N)

	Mean relative abundance (%)		M (P_{25}, P_{75})			
	Group N	Group P	Group N	Group P	*Z*	*P*
Phylum						
Actinobacteria	2.84	0.89	1.15 (0.32–2.46)	0.44 (0.11–0.80)	−2.879	0.004
Proteobacteria	7.01	8.35	3.88 (2.61–6.17)	5.98 (3.96–9.15)	−2.161	0.031
Genus						
Bifidobacterium	2.66	0.69	1.01 (0.26–2.42)	0.28 (0.05–0.67)	−2.668	0.007
Lactobacillus	0.07	0.04	0.005 (0–0.03)	0.001 (0–0.04)	−2.074	0.038
Butyricicoccus	0.003	0.001	0 (0–0.003)	0 (0–0)	−2.492	0.013
Coprobacillus	0.0008	0	0 (0–0)	0 (0–0)	−2.050	0.042
Allobaculum	0.0002	0	0 (0–0)	0 (0–0)	−2.050	0.042

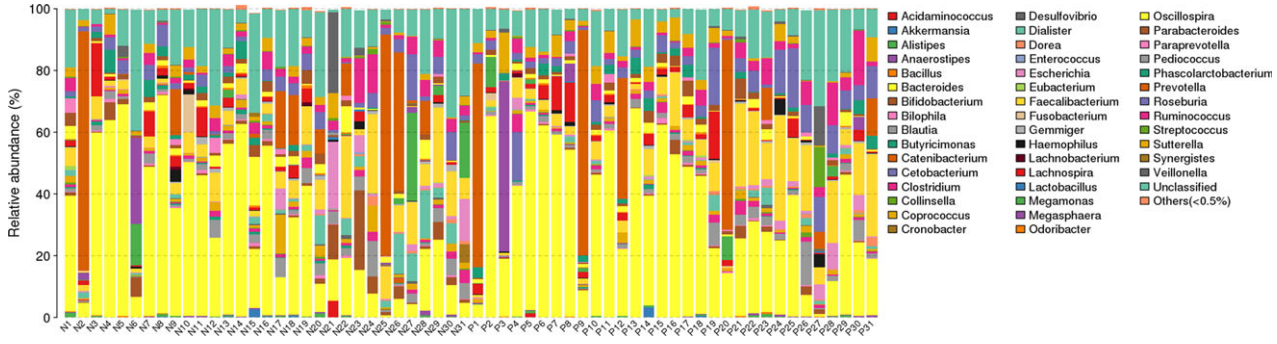

Figure 6. Relative abundance of different bacteria at genus level in each sample.

Escherichia, *Faecalibacterium*, *Lachnospira*, *Megamonas*, *Oscillospira*, *Megasphaera*, *Parabacteroides*, *Sutterella*, *Poseburia*, *Phascolarcobacterium*, *Ruminococcus*, *Veillonella* and *Bifidobacterium* were the main genera (>1%) in both groups (Fig. 6). In group N, *Bacteroides* (29.93%) and *Prevotella* (8.93%) were the two commonest genera. In group P, *Bacteroides* (35.67%) were also the commonest genera, but the second commonest genera were *Faecalibacterium* (9.58%). The proportions of *Bifidobacterium*, *Butyricicoccus*, *Coprobacillus*, *Lactobacillus* and *Allobaculum* were significantly different between the two groups (Tables 3,S2).

DISCUSSION

There were few studies about the changes in gut microbiota in acne patients. One investigation of this issue was a

Russian study in 2001, which reported different intestinal microflora in 54% of 114 acne vulgaris patients.[3] However, studies for gut microbiota at that time relied on microbiological culture technique and only 10–30% of gut microflora can be obtained.[16] Over the past few years, the development of high-throughput sequencing technology has led to great discoveries about the microbial community in the human gut. Here, we used this advanced sequencing technology based on the 16S rRNA sequences in microbial genomes to analyze the gut microbiota in 31 acne patients using 31 paired healthy individuals as the controls.

In healthy controls, the commonest phylum was Bacteroidetes, followed by Firmicutes and Proteobacteria, consistent with other studies from Chinese fecal samples[15,17] but different from the results from Americans and Egyptians, in which the commonest phyla were Firmicutes and then Bacteroidetes.[14] The major taxa are related to human colon environment, and the ingredients of diets in different countries may affect the structure of gut microbiota.[18] Diets containing large quantities of protein and animal fat lead to the dominance of Bacteroidetes, while a long-term diet rich in carbohydrates favors mainly Firmicutes.[19] In American fecal samples, the commonest phylum was Firmicutes followed by Bacteroides, and the overall Firmicutes : Bacteroides (F/B) ratio was 1.4.[14] In Egyptian samples, the F/B ratio was 1.1.[14] In our control samples, the commonest phylum was Bacteroides followed by Firmicutes, and the F/B ratio was 1. Remarkably, the F/B ratio was 0.8 in our acne patients. This ratio can be potentially used as a biomarker for pathological conditions.[20] Therefore, there may be unbalanced gut microbiota in our acne patients.

Although the bacterial types and the proportions among these types in acne patients are roughly similar to those in the controls, there are several important differences in gut microbiota in acne patients. First, the distribution of Proteobacteria and Actinobacteria phyla, the two major populations in the human gut, is significantly different between acne patients and controls. The Proteobacteria phylum is higher in acne patients. Many pathogenic bacteria including *E. coli*, *Salmonella* and *Vibrio cholerae*[21] are classified as Proteobacteria phylum, but we cannot further identify these specific species belonging to this phylum in the two groups of samples. Second, a striking underrepresentation of *Bifidobacterium*, *Butyricicoccus*, *Coprobacillus*, *Lactobacillus* and *Allobaculum* genera is found in acne patients (Table 3). *Bifidobacterium* are Gram-positive and non-spore-forming bacilli belonging to Actinobacteria phylum. *Lactobacillus* are also Gram-positive and non-spore-forming bacilli of Firmicutes phylum. The most commonly used and studied species of probiotics belong to the genera of *Lactobacillus*, *Bifidobacterium* and *Saccharomyces.*[22] They can balance intestinal microbiota by fermenting oligosaccharides not digested and absorbed in the upper gastrointestinal tract and by adversely affecting other bacteria,[23] inhibit a wide range of pathogenic microorganisms *in vitro* and *in vivo*,[24] and exert direct effects on intestinal epithelial barrier function evidenced by decreased intestinal permeability and enhanced intestinal epithelial resistance.[25] Besides, *Bifidobacterium* and *Lactobacillus* can induce the generation of regulatory dendritic cells and $CD4^{+}Foxp3^{+}T$ cells (regulatory T cells), and abundance of these regulatory cells results in hyporesponsiveness of B cells and T helper cells without apoptosis along with the suppression of cytokine production.[26] Likewise, the deleterious effects of tumor necrosis factor-α and γ-interferon on epithelial permeability and ion transport can be prevented by these bacilli,[27] helpful for the recovery from several inflammatory disorders. Several studies have demonstrated an increase in immunoglobulin A antibody production in response to *Bifidobacterium* supplementation and changes of cell-mediated immunity including antigen presentation in response to both *Bifidobacterium* and *Lactobacillus* supplementation.[28–30] *Butyricicoccus* generates butyrate. *Butyricicoccus* was significantly lower in our acne patients than healthy controls, resulting in the decrease in butyrate concentration in the gut. Butyrate produces energy in normal cells and has the effects on protection of mucosal barrier function and inflammation.[31] In particular, butyrate is a major substrate for epithelial cells to promote differentiation of colorectal cancer cells,[32,33] modulate inflammation pathways to achieve anti-inflammation effects and enhance overall gut barrier intergrity.[34,35] In addition, we found *Coprobacillus* and *Allobaculum* were significantly lower in acne patients than in healthy controls. Also, there are scarce resources relating to the roles of *Coprobacillus* and *Allobaculum* in the human gut for the explanation of these changes in acne patients.

Thus, we speculate that the changes of gut microbiota and decrease in butyrate production lead to the dysfunction of intestinal epithelial barrier and anti-inflammation mechanisms, through which acne vulgaris is aggravated. However, the exact mechanisms are still unclear and need to be further investigated. Several limitations of our study should be acknowledged. First, the information about the subtype of acne vulgaris was incomplete. Second, gut microbiota can be influenced by many factors such as lifestyle and nutrition. We only consider the relationship between gut microbiota changes and acne vulgaris, and other factors also have the possibility to affect the composition of gut microbiota. Third, this study included a small study population of Chinese subjects, preventing extrapolation of the current study to a broader scale. Finally, we performed high-throughput sequencing for V4 hypervariable regions of the 16S rRNA gene from gut fecal samples, but this technology is unsuitable for species identification. Nevertheless, this study takes advantage of high-throughput technology; the results disclose the association between acne vulgaris and gut microbiota. Research on gut microbiota in acne vulgaris in more detail will help us further understand the implications of microbiota as disease-related agents.

In summary, we identified a link between acne vulgaris and changes of gut microbiota. Increased understanding of the etiology of acne vulgaris contributes to the development of novel therapeutics for this challenging condition.

CONFLICT OF INTEREST: None declared.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

Table S1. Relative abundance (%) of the four major phyla in each individual

Table S2. Relative abundance (%) of the five genera in each individual

Table S3. Operational taxonomic unit (OTU) numbers of the four major phyla and five genera in each individual