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Gender-Specific Differences in Gut Microbiota Composition Associated with Microbial Metabolites for Patients with Acne Vulgaris

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Background: The gut microbial dysbiosis and gender differences in the pathogenesis of acne vulgaris have long been postulated respectively. However, there was no data about a gender-related discrepancy in gut microbiota and microbial metabolism in acne.
Objective: This study aimed at identifying the underlying gender-related difference in gut microbiota and metabolism in acne vulgaris.
Methods: Fecal samples were collected from 43 acne patients and 43 age and gender-matched controls. Gut microbiota was analyzed by sequencing the V3-V4 region of *16SrDNA* gene and microbial metabolites were quantitatively detected using gas chromatography time-of-flight mass spectrometry.
Results: Compared with healthy controls, the men had a lower abundance of 18 microbes such as *Butyricicoccus*, *Clostridium sensu stricto*, *Faecalibaculum*, *Bacillus*, *Lactococcus*, *Blautia*, *Clostridiales*, *Lachnospiracea incertae sedis*, *Ruminococcus* at genus level. However, the female patients only showed increased *Clostridium sensu stricto* and declined *Oscillibacter* and *Odoribacterin*. Additionally, the disordered metabolism of fatty acids was identified in male patients, while the dysbiosis of amino acids metabolism in female ones.
Conclusion: The disorder of gut microbiota and metabolism in acne vulgaris was gender-specific, which supported the potential role of gender difference in the pathogenesis of this disease.

Keywords: Acne vulgaris, Gastrointestinal microbiome, Gender differences, Metabolites

INTRODUCTION

Acne vulgaris is a common inflammatory dermatosis of the pilosebaceous units, affecting 85% of adolescents and young adults aged between 12 to 25 years[1]. Although acne prevalence among adolescents is comparable across gender, with a man to woman ratio of about 1/1.1~1.25 in Asians, acne is much more common in adult women than in adult men and it can be more severe in men than in women[2,3]. Recently, there has been an increasing interest in the gender differences both in the pathogenesis and treatment of acne vulgaris. The facial sebaceous glands in Asian skin have been detected by three-dimensional ultrasound microscopy in a study, which observed cauliflower-shaped sebaceous glands in men while more cylindrical and smaller sebaceous glands in women than the young men[4]. CAG polymorphism in the androgen receptor gene of female acne patients was more associated with nodulocystic acne, comparing with it in man cases[5]. Even, the serum metabolomic profile in patients with acne vulgaris was gender specific[6]. In clinical practice, hormone-based treatment has been always suggested to women patients rather than to men cases. Additionally, increasing studies have reported the gender-related difference in responses to the treatment of acne vulgaris[7,8].

Recently, it has been recognized that gut microbiota dysbiosis was not just a marker but also contributed to disease pathology[9,10]. Our previous study reported the existence of a

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gut microbial dysbiosis in patients with acne vulgaris, which was characterized by less microbial diversity and decreased Firmicutes/Bacteroidetes ratio[11]. In addition, Yan et al.[12] found a decrease in *Lactobacillus*, *Bifidobacterium*, *Butyricicoccus*, *Coprobacillus*, and *Allobaculum* in acne patients compared with controls, which provided a new understanding of the link between acne and the alteration of gut flora. However, it remains unclear whether the dysbiosis of gut microbiota and its associated metabolism in patients with acne vulgaris were gender-specific.

In this study, we aim to investigate the discrepancies of gut microbes and associated metabolites between men and women acne patients, which may help explain the gender-related pathogenesis and provide potential therapeutic targets of acne vulgaris.

MATERIALS AND METHODS

Sample collection

All participants were middle-school or college students in Luzhou City, Sichuan province between August 2016 and May 2017, including 43 subjects with acne vulgaris and 43 age- and gender-matched healthy controls, which were described in our previous studies[11]. The severity of acne was determined according to the Japanese Acne Study Group criteria[13], which was defined as mild (S1), moderate (S2), severe (S3), and very severe (S4) according to the number of open and closed comedones, papules, pustules, cysts and nodules on half of the face. The participants were divided further into four different groups according to gender: defined as woman acne set (FAS), woman control set (FCS), man acne set (MAS), and man control set (MCS). The participants had not used antibiotics, glucocorticoids, immunosuppressive drugs, or herbal medicines within the past 6 months and did not present with other dermatoses, obesity, infections, tumors, mental diseases, immunodeficiency, or any other systemic disorders. Fresh fecal samples were collected in a sterile container and frozen within 30 minutes at −80℃ until they were processed. All participants provided written informed consent for the use of data and samples for scientific purposes. This study was approved by the Ethical Committees of the Affiliated Hospital of Southwest Medical University (KY2019139).

16S amplicon preparation, sequencing, processing, and analysis

Microbial DNA was extracted from fecal samples using the QIAamp DNA stool Mini Kit (Qiagen Ltd., Strasse, Germany) following the manufacturer's instruction. Amplification and sequencing of the V3-V4 *16S rDNA* gene region was performed as described previously[1]. The raw 16S data were processed by USEARCH to form operational taxonomic units (OTUs) at a 3% dissimilarity level. Bacterial taxonomy assignment was performed using the RDP database and classifier (http://rdp.cme.msu.edu). Measures of α-diversity (Simpson diversity index, Shannon diversity index) among groups were calculated based on the rarefied OTU counts. Principle component analysis (PCA) was performed on OTU in order to explore the natural distribution of the four group samples. Statistical analyses of the differences in gut microbiota among the four groups were performed by Wilcoxon test and Kruskal–Wallis test using R3.1.0 (R Foundation for Statistical Computing, Vienna, Austria). All p-values reported are two-sided, and $p<0.05$ was considered to be statistically significant. We also applied the Benjamin and Hochberg false discovery rate test (FDR) or calculated the 95% confidence intervals (CI) if the FDR q value was >0.1.

Sample preparation for metabolites

Targeted quantitative analysis of 118 gut microbiome metabolites, including short-chain fatty acids (SCFAs), amino acids, carboxylic acids, benzoic acid derivatives, phenols, and indoles, was performed using the MicrobioMET platform (Metabo-Profile, Shanghai, China) with previously published methods[14,15]. Briefly, the frozen fecal samples were kept cool on a salt-ice bath, and approximately 50 mg of feces was homogenized with 300 μl of NaOH (1 M) solution followed by 200 μl of cold methanol using a homogenizer (BB24; Next, Advance Inc., Averill Park, NY, USA). The supernatant from the extractions was combined and capped in an autosampler vial. The derivatization with methyl chloroformate and injection was performed with a robotic Multi-Purpose Sampler (MPS2) with dual heads (Gerstel, Muehlheim, Germany).

Instrumentation for metabolite measurements

An Agilent 7890B gas chromatograph coupled with a GC-TOFMS system (Pegasus HT; Leco Corp., St. Joseph, MO, USA) operated in electron ionization (EI) mode was used to quantitate microbial metabolites in this project. A Rxi-5 ms

capillary column (30 m×250 μm i.d., 0.25-μm film thickness; Restek Corporation, Bellefonte, PA, USA) was used for metabolite separation. The temperature program was set at 45°C for 1 minute, increased to 260°C at 20°C/min, reached 320°C at 40°C/min, and remained at 320°C for 2 minutes. Helium was used as the carrier gas at a constant flow rate of 1.0 ml/min. The temperature of the injection and transfer interface were both set to 270°C. The measurements were made using electron impact ionization (70 eV) in the full scan mode (m/z 50-500). Instrument optimization was performed as needed.

Metabolomic data analysis

The raw data generated by GC-TOFMS were processed using ADAP software[16] for automatic baseline denoising, smoothing, peak picking, and peak signal alignment. Before the statistical analysis, we applied the Shapiro–Wilk test to examine the distribution of each continuous variable, including clinical characteristics, microbiomes, and microbial metabolomes. As a result, over 90% of the variables deviated from normal distribution; thus, non-parametric tests were used in this study. The Kruskal–Wallis test was used to compare the differences in metabolites among the four groups, including FAS, FCS, MAS, and MCS. Next, we used the Mann–Whitney U test to compare the difference of each metabolite between two sample sets, such as MAS and MCS groups. Variables with *p*-values smaller than 0.05 were considered to be statistically significant. In addition, we calculated Spearman's rank correlation coefficients to measure the relationships between metabolome and microbiome, which were further visualized using heat map analysis to indicate their positive or negative correlations.

RESULTS

Study cohorts

A total of 86 participants were divided into four sub-groups according to health state and their gender, their demographic information of the participants is summarized in Table 1. Gender and age between patients and healthy controls were matched. There was no difference in body mass index ($p=0.225$) among the four groups in this study. Women with acne vulgaris were more elderly than the men cases ($p=0.02$), but all of them were less than 25 years old and could be diagnosed as adolescent acne. Significantly, the men had more serious acne, compared with women ($p<0.001$). However, we have found no significant difference in bacterial diversity and structures among patient subgroups with different severity[11].

Gender-based comparison in diversity and structure of gut microbiota between patients with healthy controls

A total of 3,989,761 clean sequencing reads that passed the Q20 filtering were obtained from the 86 fecal samples, and the average length of base pairs was 416. Compared with healthy controls, microbial diversity was significantly decreased in MAS, as calculated by the Shannon diversity index ($p=0.048$) and Simpson diversity index ($p=0.047$; Fig. 1A, B). However, no significant difference in microbial diversity was observed between FAS patients and the controls. ANOSIM analysis indicated the significance of clustering samples among the four groups (R=0.057, $p=0.019$) (Fig. 1C). PCA scores plot of four groups based on OTUs indicated that MCS was the most different group from the other three groups, and MAS and MCS were separated while FAS and FCS groups were close to each other (Fig. 1D). Multi-response permutation procedure analysis indicated a significant difference among each set ($p=0.044$, data not shown).

Gender-specific bacterial taxa differences between patients with acne vulgaris and healthy controls

The majority of OTUs were assigned to four main phyla: *Bacteroidetes*, *Firmicutes*, *Proteobacteria*, and *Actinobacteria*, which

Table 1. Summary of demographic information of study participants

Name	MAS	FAS	MCS	FCS	*p*-value
Number	26	17	26	17	-
Age (yr)	19.31±2.45	21.00±2.98	19.31±2.45	21.00±2.98	0.015
BMI (kg/cm^2)	21.08±1.78	19.70±1.51	21.41±5.24	20.16±2.05	0.225
Disease severity (S1, S2, S3, S4)	2, 9, 7, 8	10, 3, 2, 2	-	-	<0.001

Values are presented as mean±standard error of mean. *p*-value was calculated from the Kruskal–Wallis test. BMI: body mass index, MAS: man acne set, FAS: woman acne set, MCS: man control set, FCS: woman control set.

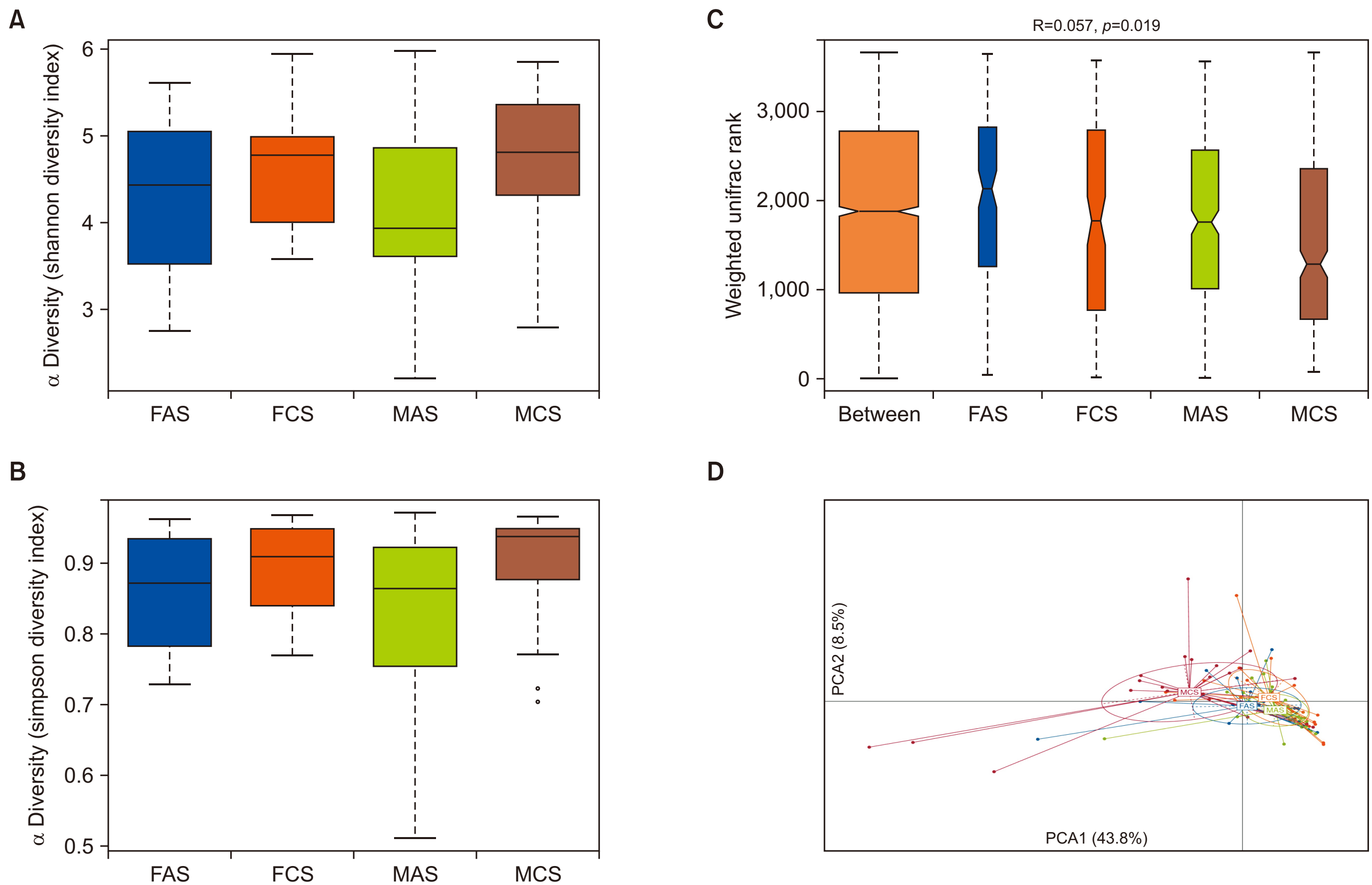

Fig. 1. Comparisons of different diversity indices and microbiota structure in four samples. Participants were separated into four groups as the MAS, FAS, and their control sets (MCS, FCS). (A) Shannon diversity index ($p=0.048$) among four groups. (B) Simpson diversity index ($p=0.047$) among four group. (C) The difference among and within groups was assessed by ANOSIM analysis. (D) PCoA plot with different relative abundances of OTU among four groups. FAS: woman acne set, FCS: woman control set, MAS: man acne set, MCS: man control set.

were also the most abundant ones in all of the subjects (Fig. 2A). The abundance of *Firmicutes* (p=0.001) and *Bacteroidetes* (p=0.002) were significantly increased and decreased in the MAS group compared to MCS respectively. However, the difference was not found between FAS and FCS groups (Fig. 2B). Then the gender-based comparison for patients and healthy people were further analyzed by Mann–Whitney test. When compared with healthy man, men with acne vulgaris had significantly lower abundance of such 18 genus as *Lysinibacillus*, *Paenibacillus*, *Aerococcus*, *Alkaliphilus*, *Carnobacterium*, *Lactococcus*, *Oceanobacillus*, *Bacillus*, *Blautia*, *Butyricicoccus*, *Gemmiger*, *Lachnospiracea_incertae_sedis*, *Exiguobacterium*, *Pseudomonas*, *Enterococcus*, *Faecalibacterium*, *Bilophila*, and *Ruminococcus*. However, the women just had increased *Clostridium sensu stricto* and declined *Oscillibacter* and *Odoribacterin* (Supplementary Fig. 1). Although the q-value >0.1 was calculated for all above genus, the 95% CIs of mean abundance difference of those taxa never spanned 0 (Supplementary Table 1).

Targeted analysis of gut microbial metabolites associated with gender in patients with acne vulgaris

Using time-of-flight mass spectrometry (GC-TOFMS) metabolomics platform, a total of 118 gut microbial metabolites were identified in fecal samples, including amino acids, fatty acids, carboxylic acids, hydroxylic acids, and phenolic acids, benzoyl and phenyl derivatives, and indoles. Multivariate orthogonal partial least squares discriminant analysis models were applied and the scores plots showed the overall differences of metabolic profiles between two groups (Supplementary Fig. 2). Then, we applied Mann–Whitney U test and identified 14 differential metabolites between the MCS and FCS groups, in-

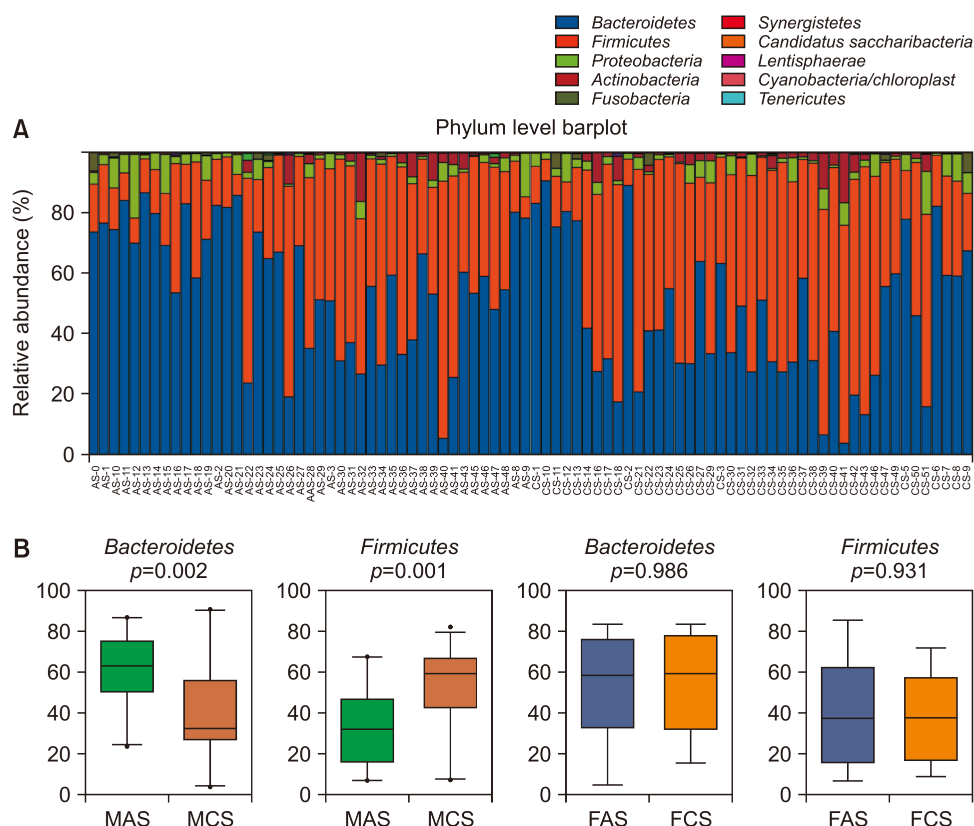

Fig. 2. The majority of microbes at phyla level in all samples, and the gender-based difference of taxa at phyla level in patients with acne vulgaris. (A) The majority of OTU were assigned to four main phyla, *Bacteroidetes*, *Firmicutes*, *Proteobacteria*, and *Actinobacteria*. (B) The comparison of the relative abundance of *Firmicutes* and *Bacteroidetes* between acne patients and their gender-matched controls. FAS: woman acne set, FCS: woman control set, MAS: man acne set, MCS: man control set.

cluding increased 2-phenylglycine, D-2-hydroxyglutaric acid, glyceric acid, Glycine, L-alanine, L-alpha-aminobutyric acid, L-cystine, L-histidine, L-methionine, L-serine, myristic acid, ornithine, putrescine and decreased 3-hydroxyisovaleric acid in man (Supplementary Table 2). In addition, we identified 14 differential metabolites between the MAS and MCS groups and 13 ones between FAS and FCS (Table 2). Men had an increased level of 3-methylindole, alpha-linolenic acid, glyceric acid, glyceric acid, L-asparagine, L-tryptophan, linoleic acid, N-acetyltryptophan, oxoglutaric acid, phenyllactic acid, purine, stearic acid, succinic acid, and only one decreased valeric acid. The concentration of serum 2-phenylglycine, 4-hydroxyphenylpyruvic acid, glycine, L-alanine, L-histidine, L-leucine, L-methionine, L-serine, L-tryptophan, L-valine, methylsuccinic acid, N-acetyltryptophan, ornithine was higher in women than them in healthy controls. We can see, the women and men patients had completely different changes of metabolites while comparing with controls. The men tended to have disordered metabolism of fatty acids, but the women's cases had dysbiosis of metabolites from amino acids.

Integrated analysis of gut microbiota and metabolites

Next, we performed the Spearman correlation analysis between differential microbes and metabolites identified by comparing gender-based patients with their controls. Their relative positive or negative relationships were visualized using heat maps (Fig. 3). Most interestingly, we observed that the metabolites of linoleic acid, alpha-Linolenic acid, oxoglutaric acid, phenylacetic acid, stearic acid, succinic acid were negatively associated with such microbes as *Lachnospiracea*, *Blautia*, *Ruminococcus*, *Gemmiger*, *Fusicatenibacter*, and *Butyricicoccus* in men with acne. For women, the metabolites of 2-phenylglycine, 4-hydroxyphenylpyruvic acid, glycine, L-alanine were positively related to *Clostridium sensu stricto* and 2-phenylglycine, L-leucine, L-methionine, L-serine, L-valine was negatively correlated with declined *Oscillibacter*.

DISCUSSION

Acne is the most common skin disease, as well as a cardinal component of many systemic diseases or syndromes[17]. Although it clearly develops from an interplay of environmental factors and genetic predisposition, the exact cause of acne

Table 2. Gender-based difference of metabolites between patients and healthy controls

Metabolites	Group		*p*-value	Z-value
	Acne set	Control set		
Man				
3-Methylindole	0.63±0.94	0.33±1.15	0.029	−2.18
Alpha-linolenic acid	39.44±48.74	35.93±20.90	0.046	−2.00
Glyceric acid	516.53±663.92	174.41±298.02	0.039	−2.07
Hydroxypropionic acid	25.61±39.27	17.23±32.45	0.039	−2.07
L-asparagine	19.07±32.05	17.35±57.91	0.037	−2.09
L-tryptophan	11.58±16.71	6.74±13.70	0.042	−2.03
Linoleic acid	124.66±120.85	62.47±52.79	0.022	−2.29
N-acetyltryptophan	21.14±31.97	12.00±26.23	0.044	−2.01
Oxoglutaric acid	48.06±57.30	17.49±29.14	0.013	−2.47
Phenyllactic acid	0.42±0.42	0.18±0.16	0.019	−2.35
Purine	1.62±2.38	1.07±1.45	0.021	−2.31
Stearic acid	11.07±7.10	7.92±6.26	0.035	−2.11
Succinic acid	28.89±31.97	13.84±18.99	0.046	−2.00
Valeric acid	4,506.14±1,841.31	5,499.25±1,502.47	0.017	−2.38
Woman				
2-Phenylglycine	2.73±2.66	0.75±0.60	0.018	−2.36
4-Hydroxyphenylpyruvic acid	1.77±1.42	0.91±0.32	0.007	−2.70
Glycine	5.51±3.43	2.77±2.01	0.008	−2.67
L-alanine	2.76±1.72	1.39±1.00	0.008	−2.67
L-histidine	6.78±3.82	3.80±3.36	0.015	−2.43
L-leucine	33.83±24.89	20.01±14.88	0.048	−1.98
L-methionine	74.60±53.23	38.71±28.07	0.022	−2.29
L-serine	12.90±7.88	8.22±6.89	0.031	−2.15
L-tryptophan	5.97±6.05	3.16±2.72	0.024	−2.26
L-valine	27.12±24.65	13.29±10.00	0.034	−2.12
Methylsuccinic acid	5.31±16.59	0.70±0.46	0.044	−2.02
N-acetyltryptophan	9.97±10.87	5.26±4.93	0.024	−2.26
Ornithine	3.21±10.37	0.34±1.13	0.031	−2.15

Values are presented as mean±standard error of mean.

remains elusive. It was increasingly believed that the gut microbiota could be involved in the pathogenic process of acne[11,12]. In addition, the previous data have indicated that disease-associated differences in gut microbiota composition, functions, and ecological networks were markedly influenced by gender[18-23]. In this study, the decreased diversity of gut microbiota was only found in the men acne samples but not in women, which also occurs in other inflammatory skin diseases[24,25]. It was shown in previous studies that people with low microbial diversity are characterized by a more pronounced inflammatory phenotype[26]. For acne vulgaris, the men tend to suffer more severe lesion[3], which could be associated with the low bacterial richness.

Besides, the lower abundance of anti-inflammation-related bacteria such as *Butyricicoccus*, *Bacillus*, *Lactococcus*, *Blautia*, *Clostridium sensu stricto*, *Faecalibaculum*, *Clostridiales*, *Lachnospiracea_incertae_sedis*, and *Ruminococcus* also linked to the greater inflammation in men with acne[3]. *Butyricicoccus*

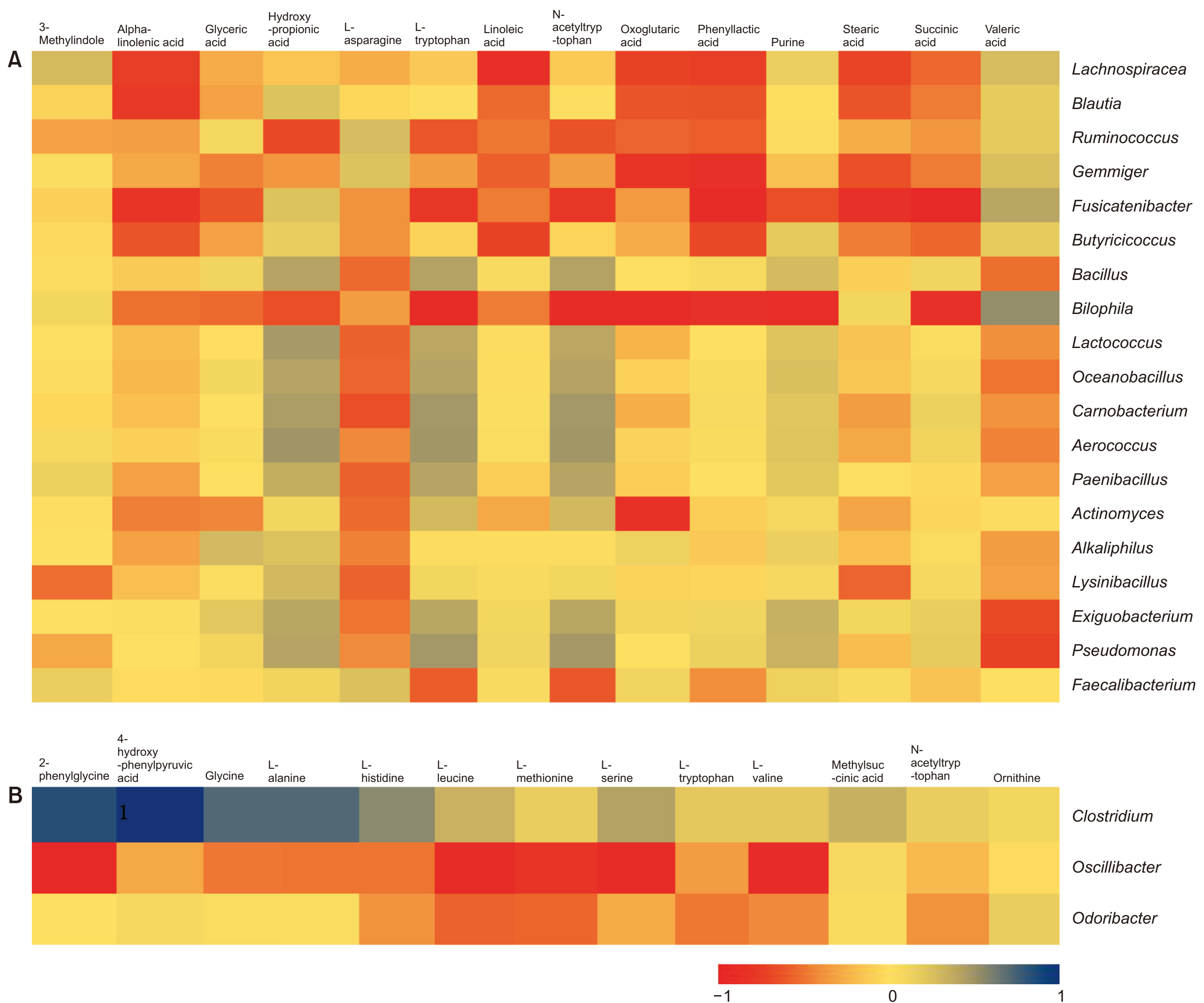

Fig. 3. Correlation between gut microbial taxa and metabolites was assessed by Person correlation test and displayed as a heat map. (A) For men patients; (B) for women patients.

generates butyrate, which provides energy to cells and prevents mucosal barrier damage and inflammation[27]. The *Clostridium sensu stricto*, *Faecalibaculum* are both SCFAs-producing bacteria[28], which plays an important role in the connection between gut and skin microbiota[29]. SCFAs were shown to have a profound antimicrobial effect against methicillin-resistant *Staphylococcus aureus*, consequently contributing to shaping the skin microbiota, which may influence cutaneous immunity[30]. *Propionibacterium acne* and *Staphylococcus epidermidis* are examples of skin commensals enduring wide SCFA shifts. In addition, the decreased *Clostridiales*, *Lachnospiracea incertae sedis*, and *Ruminococcus* were also found in men with acne vulgaris, which have been reported to be less abundant in immune and inflammatory diseases[11,31]. Furthermore, the man patient group showed several decreased genera taxa belonging to *Bacilli* class, such as *Lactococcus*, *Carnobacterium*, *Aerococcus*, *Lysinibacillus*, *Bacillus*, and *Oceanobacillus*. *Bacilli* is known to produce a vast array of antimicrobial compounds, such as 3-hydroxypropionaldehyde and common probiotics[32,33]. Recent studies reported the declined abundance of beneficial bacteria, *Lactococcus*, *Bacillus*, *Clostridium* in correlation with the inhibition of the mTOR pathway, the key signaling for pro-

moting the proliferation and secretion of sebaceous glands[34,35]. Finally, our study found the significantly rising long-chain saturated fatty acids, Alpha-linolenic acid, linoleic acid, stearic acid, while the declining SCFA valeric acid in men. Linoleic acid, an important omega-6 fatty acid, has been reported to be a pro-inflammatory substance and even aggravate the severity of acne vulgaris[36,37]. A previous study showed that a large number of these unsaturated fatty acids, alpha-linolenic acid, linoleic acid, were parallel to the down-regulation of the PI3K/Akt/mTOR pathway[38]. However, further studies should be performed to identify how the disorder of gut microbiota and its associated metabolites contribute to the pathogenesis of man acne through modulating mTOR signaling.

A cohort study including 4,7111 patients with acne vulagris found that woman gender was independently and jointly associated with major depression and suicide[39]. In this study, the increased microbes of *Clostridium sensu stricto* and declined *Oscillibacter* and *Odoribacterin* were identified in women acne patients, which also has been reported to be associated with psychological stress[40]. In the aspect of metabolism, microbial metabolites from aminoadipic acids, such as L-alanine, L-histidine, L-leucine, L-methionine, L-serine, L-tryptophan, L-valine, were increased in the FAS group. The dysbiosis of aminoadipic acid metabolism has been reported to be associated with an increased risk of stress-related psychiatric illnesses[41]. This study also found the positive correlation of L-leucine, L-methionine, L-serine, L-valine with the relative abundance of *Oscillibacter*, a depression-associated bacteria[42]. In 1930, Stokes and Pillsbury postulated that psychological stress causes intestinal microbes to produce neurotransmitters that cross the intestinal mucosa to enter the bloodstream, resulting in systemic inflammation[16]. The theory of the microbiota-gut-brain axis may further support the mechanism of gut microbiota underlying the pathogenesis of acne vulgaris in women.

In conclusion, this study demonstrated for the first time that men and women acne vulgaris patients have significantly different dysbiosis of gut microbiota and associated metabolites. However, there were some limitations of this study. Firstly, we detected 145 gut microbiome metabolites but did not perform micro metabolomics analysis; however, the measurement of these products was quantitative. Additionally, the sample size of adolescent acne was limited. But in this study, all participants had not been treated with antibiotics for at least 6 months and were age- and gender-matched, which could consolidate the result. Lastly, the study was descriptive and the causal relationship of gut microbiota and acne was not shown.

SUPPLEMENTARY MATERIALS

Supplementary data can be found via http://anndermatol.org/src/sm/ad-33-531-s001.pdf.

CONFLICTS OF INTEREST

The authors have nothing to disclose.

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DATA SHARING STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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