

## 论著·基础研究

## 中度与重度寻常痤疮患者非皮损区毛囊菌群分析：一项单中心横断面研究

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**[摘要]** **目的**·研究中度、重度寻常痤疮患者与健康者非皮损区毛囊菌群群落结构和载量的差异, 探讨微生物与寻常痤疮及其严重程度之间的关系。**方法**·采用横断面研究, 选取2022年8月—2023年8月在上海交通大学医学院附属仁济医院皮肤科就诊的中度、重度寻常痤疮 (简称痤疮) 患者和健康志愿者。取中度、重度痤疮患者及健康志愿者面部非皮损区毛囊内容物进行16S rRNA高通量测序以及实时荧光定量聚合酶链反应 (quantitative real-time polymerase chain reaction, qPCR) 检测, 分析不同严重程度痤疮患者毛囊细菌菌群多样性、物种组成差异和菌群载量差异。**结果**·纳入10例中度痤疮患者、11例重度痤疮患者及11例健康志愿者, 3组间年龄、性别等一般资料差异均无统计学意义。中度痤疮组和重度痤疮组相对于健康组细菌 $\alpha$ 多样性均显著下降 ( $P=0.020$ ,  $P=0.013$ )。主坐标分析 (principal coordinates analysis, PCoA) 图表明健康组人群样本分布较为集中, 组内差异较小, 而中度痤疮组及重度痤疮组人群样本分布有一定的趋势但较为离散, 组内之间存在较大差异。3组样本趋势分布差异明显, 组间微生物群落结构差异较大。相似性分析结果显示健康组与中度痤疮组 ( $P=0.027$ )、健康组与重度痤疮组 ( $P=0.017$ ) 组间 $\beta$ 多样性有显著差异, 物种组成相似度低。中度痤疮组与重度痤疮组 ( $P=0.160$ ) 组间物种相似度较高。门水平上3组的优势菌群均为放线菌门 (Actinobacteria)、厚壁菌门 (Firmicutes)、变形菌门 (Proteobacteria) 以及拟杆菌门 (Bacteroidetes)。属水平上健康组优势菌为丙酸杆菌属 (*Propionibacterium*)、未分类的放线菌属 (unclassified *Actinomycetales*), 2组痤疮组优势菌均为葡萄球菌属 (*Staphylococcus*)、丙酸杆菌属。相对于健康组, 中度痤疮组和重度痤疮组非皮损区毛囊中的葡萄球菌属相对丰度均显著增加 ( $P=0.010$ ,  $P=0.019$ ), 毛囊菌群载量均显著增加 (均  $P=0.001$ )。与中度痤疮组相比, 重度痤疮组毛囊样本中细菌载量显著升高 ( $P=0.017$ )。**结论**·中度、重度痤疮患者与健康者非皮损区毛囊微生物群落结构不同, 痤疮组微生物多样性明显下降。中度痤疮组及重度痤疮组非皮损区毛囊中的葡萄球菌属相对丰度较健康组均显著增加。随着痤疮严重程度的增加, 非皮损区毛囊内细菌载量显著增加。研究提示痤疮的发生以及严重程度可能与毛囊菌群的群落结构和载量有关。

**[关键词]** 寻常痤疮; 毛囊菌群; 葡萄球菌属; 细菌载量**[DOI]** 10.3969/j.issn.1674-8115.2024.09.004 **[中图分类号]** R751 **[文献标志码]** A

## Analysis of hair follicle microbiota in non-lesional areas of patients with moderate-to-severe acne vulgaris: a single-center cross-sectional study

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**[Abstract]** **Objective**·To study the differences in the structure and load of hair follicle microbiota in non-lesional areas among patients with moderate-to-severe acne vulgaris and healthy individuals, and to explore the relationship between microorganisms and the severity of acne vulgaris. **Method**·A cross-sectional study was used. Patients with moderate or severe acne vulgaris (referred to as acne) and healthy volunteers who visited the Department of Dermatology, Renji Hospital, Shanghai Jiao Tong University School of Medicine, from August 2022 to August 2023. 16S rRNA high-throughput sequencing and quantitative real-time polymerase chain reaction (qPCR) were performed on the follicular contents from the non-lesional areas of the faces of patients with moderate or severe acne and healthy volunteers to analyze the diversity, species composition, and microbial load differences in hair follicle bacteria in patients with different severity of acne. **Results**·Ten patients with moderate acne, eleven patients with severe acne, and

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eleven healthy volunteers were included. There were no statistically differences in general data such as age and gender ratio among the three groups. Bacterial  $\alpha$ -diversity was significantly lower in both the moderate and severe acne groups compared to the healthy group ( $P=0.020$ ,  $P=0.013$ ). The principal coordinates analysis (PCoA) plot showed that the sample distribution of the healthy group was relatively concentrated, with small differences within the group, and the distribution of samples in the moderate and severe acne groups exhibited a certain trend but was relatively scattered, with differences between the groups. There were differences in the trend distribution of the three sample groups, and there were differences in the microbial community structure between the groups. The results of similarity analysis showed significant differences in  $\beta$ -diversity and low similarity in species composition between the healthy and moderate acne groups ( $P=0.027$ ) and between the healthy and severe acne groups ( $P=0.017$ ), and high species similarity between the moderate acne and severe acne groups ( $P=0.160$ ). The dominant bacterial groups at the phylum level were Actinobacteria, Firmicutes, Proteobacteria, and Bacteroidetes. At the genus level, the dominant bacteria in the healthy group were *Propionibacterium* and unclassified *Actinomycetales*, and the dominant bacteria in both acne groups were *Staphylococcus* and *Propionibacterium*. Compared to the healthy group, the relative abundance of *Staphylococcus* species in the hair follicles in non-lesional areas of the moderate and severe acne groups was significantly increased ( $P=0.010$ ,  $P=0.019$ ). Compared with the healthy control group, the hair follicle microbiota load in non-lesional areas of both the moderate and severe acne groups was significantly increased (both  $P=0.001$ ). Compared with the moderate acne group, the bacterial load in the hair follicle samples of the severe acne group was significantly increased ( $P=0.017$ ). **Conclusion** The microbial community structure of hair follicles in non-lesional areas of patients with moderate or severe acne is different from that of healthy individuals, and the microbial diversity in the acne group is significantly reduced. The relative abundance of *Staphylococcus* species in the hair follicles in non-lesional areas of the moderate or severe acne groups is significantly increased compared to the healthy group. As the severity of acne increases, the bacterial load in hair follicles in non-lesional areas significantly increases. This research suggests that the occurrence and severity of acne may be related to the community structure and load of hair follicle microbiota.

**[Key words]** acne vulgaris; hair follicle microbiota; *Staphylococcus* genus; bacterial load

寻常痤疮（简称痤疮）是一种常见的毛囊皮脂腺的慢性炎症性疾病，影响着全球约9%的人口和85%的青少年<sup>[1]</sup>。痤疮的临床特征是皮脂溢出、炎性皮损（丘疹、脓疱、结节和囊肿）、非炎性皮损（被称为“黑头”的开放性粉刺和被称为“白头”的闭合性粉刺）<sup>[2]</sup>，常见的后遗症表现为瘢痕以及色素沉着。这些特征可能会导致焦虑、抑郁和自卑，从而影响患者的精神状态和社交活动<sup>[3]</sup>。

痤疮的病因和发病机制目前仍不清楚。越来越多的研究表明，微生物群失衡在痤疮的致病中起到至关重要的作用<sup>[4]</sup>。目前一些对痤疮微生态的研究对比了痤疮患者与健康人群皮肤表面菌群的差异：一项研究<sup>[5]</sup>表明，痤疮组与健康组皮肤微生物多样性无明显差异，假单胞菌、雷氏菌和假丝酵母以及念珠菌丰度发生明显改变；而另一项研究<sup>[6]</sup>提示痤疮患者皮肤表面微生物多样性大于健康对照，乳球菌属相对丰度增加。不同测序结果的差异可能与取材地点的气候、时间以及取材部位等多种因素有关。与皮肤表面不同，毛囊皮脂腺单位是一个相对密闭、缺氧且富含脂质的环境，其中的微生态结构与皮肤表面有所差异，并且受外界环境因素的影响较小，似乎与痤疮的发生更加密切。一项来自中国成都的观察性研究<sup>[7]</sup>比较了痤疮患者非炎性皮损和炎性皮损的菌群组成和差异，结果显示2组毛囊微生物 $\alpha$ 多样性差异无统计学意义。

尽管有关痤疮微生态的研究已有不少，但关于痤疮与微生物的关系仍存在许多未知。在痤疮发生的最早期阶段，毛囊漏斗部角化过度并引起毛囊皮脂腺导管堵塞，形成肉眼无法观察到的微粉刺。实际上，健康人群皮肤表面也可出现微粉刺，但为何只有痤疮患者会进一步发展出现后续丘疹、脓疱等皮损？此外，重度痤疮患者的皮损中存在着毛囊壁的破裂，当炎症进一步扩大时，导致结节、囊肿甚至后续瘢痕的形成，而中度痤疮患者皮损中则较少出现这样的情况，是何因素造成了痤疮皮损不同的发展结局？目前尚未有研究关注。因此，本研究拟通过16S rRNA高通量测序以及实时荧光定量聚合酶链反应（quantitative real-time polymerase chain reaction, qPCR）分析，阐明中度、重度痤疮患者非皮损区毛囊（即微粉刺）中的微生态差异。

## 1 对象与方法

### 1.1 研究对象

本研究为横断面研究，选取2022年8月—2023年8月在上海交通大学医学院附属仁济医院皮肤科就诊的中度、重度痤疮患者和健康志愿者。痤疮组纳入标准：①年龄18~30岁。②3个月内未使用过任何抗痤疮药物、物理治疗或医学美容治疗，未使用过口服

或外用抗生素。③ Pillsbury 分级<sup>[8]</sup> 为 2~3 级的中度痤疮患者或 Pillsbury 分级为 4 级的重度痤疮患者。健康对照组为 18~30 岁健康体检者。研究对象的排除标准：① 近 3 个月使用过任何抗痤疮药物、物理治疗或医学美容治疗，以及口服或外用抗生素。② 存在其他面部疾病；有严重的心脑血管、肝、肾、自身免疫、肿瘤、血液、内分泌、神经、精神疾病和/或免疫缺陷。③ 参加其他临床试验研究者。

1.2 研究方法

1.2.1 标本收集 受试者清水洁面后端坐于治疗床上，操作者佩戴口罩、帽子、手套，70%的乙醇消毒整个鼻头 3 次，鼻头贴（花王，日本）粘贴于鼻部 20 min 后缓慢揭下，用高压灭菌后的镊子在立体显微镜下挑取 30 个毛囊内容物，均匀混悬于 200  $\mu$ L 灭菌生理氯化钠溶液（上海上药信谊药厂有限公司）中，并放置于-80℃冰箱保存。

1.2.2 DNA 提取和高通量测序 使用细菌基因组提取试剂盒 [天根生化科技（北京）有限公司] 提取全基因组 DNA。用于 PCR 扩增的引物序列如表 1 所示，由上海华大基因科技有限公司合成。扩增产物在 Illumina MiSeq 平台 PE300（因美纳，美国）上测序。

表 1 引物序列  
Tab 1 Primer sequences

Primer name	Primer sequence (5'→3')	Amplified region
341F	CCTACGGGNGGCWGCAG	V3-4 <sup>[9]</sup>
805R	GACTACHVGGGTATCTAATCC	

1.2.3 qPCR 研究中所用的引物序列同表 1，使用 ViiA7 实时荧光定量 PCR 系统（Applied Biosystems，美国）进行 qPCR 反应，扩增条件见表 2。将已知拷贝数的标准品（上海华大基因科技有限公司）按照浓度梯度稀释法（以 10<sup>-1</sup> 为阶梯）进行稀释，并与待检测样本在相同条件下进行 qPCR 检测，每个样本设置 3 个重复孔，得到标准曲线公式。根据其 C<sub>t</sub> 值和标准曲线公式，得到每个样本中的细菌拷贝数。

表 2 qPCR 绝对定量程序  
Tab 2 qPCR absolute quantification program

Step	Program
Step 1	95 °C 30 s
Step 2 (40 cycles)	95 °C 5 s; 55 °C 30 s; 72 °C 30 s
Step 3 (dissociation stage)	94 °C 30 s; 70 °C 1.5 min; 94 °C 10 s

1.3 统计学分析

使用 GraphPad Prism 9.0 软件进行统计学分析和作图。定性资料以频数（百分率）表示，多组比较采用  $\chi^2$  检验。定量资料采用  $\bar{x} \pm s$  表示；符合正态分布的定量资料，采用独立样本 *t* 检验进行组间比较；不符合正态分布的定量资料，采用 Mann-Whitney *U* 检验进行组间比较；采用 Kruskal-Wallis 检验进行多组数据比较。对  $\beta$  多样性进行基于 bray-curtis 距离的主坐标分析（principal coordinates analysis, PCoA）和 Anosim 相似性分析。将线性判别分析（linear discriminant analysis, LDA）值设为 2 时，进行 LEfSe（LDA effect size）分析。*P* < 0.05 表示差异具有统计学意义。

2 结果

2.1 一般资料

共纳入 10 例中度痤疮患者（M 组）、11 例重度痤疮患者（S 组）和 11 例健康志愿者（C 组）。一般资料见表 3，差异无统计学意义。

2.2 细菌 V3~4 区域高通量测序结果

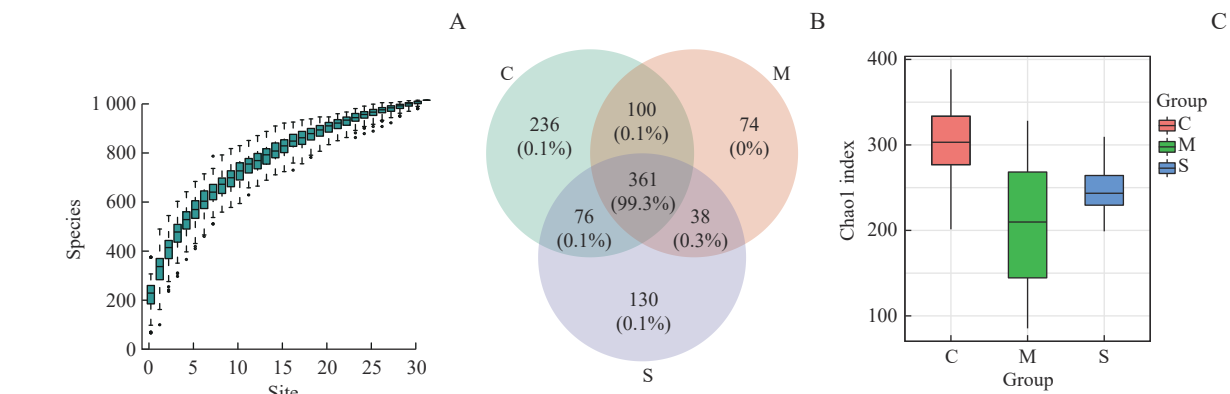
2.2.1  $\alpha$  多样性分析 本研究中随着测序样本量的增加，物种累积曲线（图 1A）趋于平缓，说明此环境中的物种并不会随样本量的增加而显著增多，表明本研究抽样充分。如图 1B 所示，C 组、M 组、S 组菌群微生物操作分类单元（operational taxonomic unit, OTU）数分别有 773、573、605 个，其中 361 个 OTU 在 3 组中相同；C 组和 M 组菌群有 461 个 OTU 相同，C 组和 S 组菌群有 437 个 OTU 相同，M 组和 S 组菌群有 399 个 OTU 相同。如图 1C 所示，C、M、S 3 组样本中代表  $\alpha$  多样性的 Chao 1 指数分别为 301.43 $\pm$ 52.82、208.55 $\pm$ 85.19、247.28 $\pm$ 30.04，C 组与 M 组之间差异有统计学意义（*P* = 0.020），即 M 组  $\alpha$  多样性指数相对 C 组显著下降；S 组相对于 C 组  $\alpha$  多样性同样显著下降（*P* = 0.013）；而 M 与 S 组之间差异无统计学意义（*P* = 0.314）。

2.2.2  $\beta$  多样性分析  $\beta$  多样性是以物种群落间的距离来评估样本间物种群落的差异程度。PCoA 图（图 2）表明 C 组人群样本分布较为集中，组内差异较小；而 M 及 S 组人群样本分布有一定的趋势但较为离散，组

表3 3组人群一般资料

Tab 3 General characteristics of the three groups

Characteristic	C group (n=11)	M group (n=10)	S group (n=11)	H/ $\chi^2$ value	P value
Gender/n(%)				0.182	0.913
Male	5 (45.5)	5 (50.0)	6 (54.5)		
Female	6 (54.5)	5 (50.0)	5 (45.5)		
Age/year	24.64±1.63	21.90±2.88	23.18±5.42	3.827	0.252
Ethnic group/n(%)				1.971	0.373
Han	10 (90.9)	10 (100.0)	11 (100.0)		
Bai	1 (9.1)	0 (0)	0 (0)		
Education/n(%)				2.676	0.262
Senior high school/technical secondary school	4 (36.4)	2 (20.0)	6 (54.5)		
Junior college and above	7 (63.6)	8 (80.0)	5 (45.5)		
Work/n(%)				1.703	0.427
In service	3 (27.3)	2 (20.0)	5 (45.5)		
Not in service	8 (72.7)	8 (80.0)	6 (54.5)		
Habitual residence/n(%)				2.147	0.342
Shanghai	11 (100.0)	9 (90.0)	9 (81.8)		
Others	0 (0)	1 (10.0)	2 (18.2)		



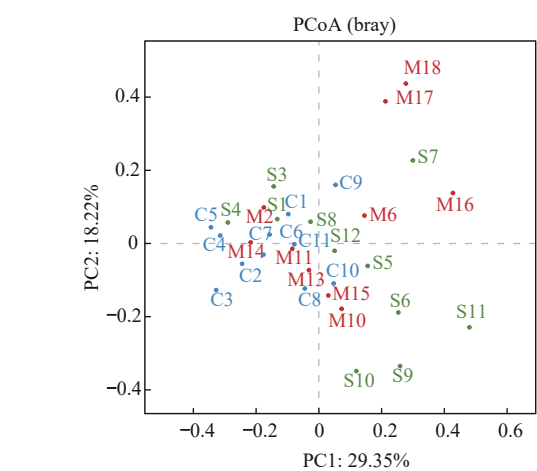
**Note:** A. Species accumulation curve. B. Venn diagrams of the OTU numbers of the three groups. C. Box plots of Chao1 index of the three groups.

图1 3组人群毛囊菌群的 $\alpha$ 多样性分析

Fig 1  $\alpha$  diversity analysis of follicular microbiome of the three groups

内之间存在较大差异;3组样本趋势分布差异明显,说明其微生物群落结构差异较大。相似性分析结果显示C组与M组( $P=0.027$ )、C组与S组( $P=0.017$ )组间 $\beta$ 多样性差异有统计学意义,物种组成相似度低;M组与S组( $P=0.160$ )组间物种相似度较高。

**2.2.3 物种组成分析** 按照门、属划分的3组人群细菌菌群的物种相对丰度见表4~5。如图3所示,门水平上3组的优势菌群均为放线菌门(Actinobacteria)、厚壁菌门(Firmicutes)、变形菌门(Proteobacteria)以及拟杆菌门(Bacteroidetes)。相对于C组,M组中变形菌门相对丰度显著降低( $P=0.043$ ),S组中厚壁菌门相对丰度显著升高( $P=0.040$ )。如图4所示,属水平上C组菌群相对丰度排名前五的菌属依次为丙酸



**Note:** PCoA scatter plot based on  $\beta$  diversity index between samples.

图2 3组人群毛囊菌群的 $\beta$ 多样性分析

Fig 2  $\beta$ -diversity analysis of hair follicle microbiome of the three groups



表 4 3 组样本细菌菌群在门水平上的丰度(%)

Tab 4 Abundance of bacterial flora at the phylum level in three sample groups (%)

Phylum	Abundance/%		
	C group (n=11)	M group (n=10)	S group (n=11)
Actinobacteria	61.48±19.39	58.48±17.59	49.64±28.26
Firmicutes	21.94±16.10	35.42±18.37	43.90±26.42
Proteobacteria	14.84±15.23	5.27±5.79	5.51±5.41
Bacteroidetes	0.86±0.88	0.27±0.29	0.59±1.13
Others	0.88±0.55	0.56±0.57	0.36±0.49

杆菌属 (*Propionibacterium*)、未分类的放线菌属 (unclassified *Actinomycetales*)、葡萄球菌属 (*Staphylococcus*)、未分类的奈瑟菌属 (unclassified *Neisseriaceae*) 以及类芽孢杆菌属 (*Caldalkalibacillus*); M组和S组菌群相对丰度排名前五的菌属均为葡萄球菌属、丙酸杆菌属、未分类的放线菌属、棒状杆菌属 (*Corynebacterium*) 以及未分类的奈瑟菌属。随着痤疮严重程度增加,丙酸杆菌属相对丰度呈现下降趋势,但差异无统计学意义;相对于

C组, M组和S组毛囊中的葡萄球菌属相对丰度均显著增加 ( $P=0.010$ ,  $P=0.019$ )。

表 5 3 组样本细菌菌群在属水平上的丰度(%)

Tab 5 Abundance of bacterial flora at the genus level in three sample groups (%)

Genus	Abundance/%		
	C group (n=11)	M group (n=10)	S group (n=11)
<i>Staphylococcus</i>	13.05±11.54	31.54±17.84	37.30±27.09
<i>Propionibacterium</i>	36.99±17.67	25.94±19.63	23.78±16.30
Unclassified <i>Actinomycetales</i>	18.78±19.20	23.38±15.96	19.58±17.88
<i>Corynebacterium</i>	2.09±2.77	8.28±11.28	5.47±4.74
Unclassified <i>Neisseriaceae</i>	4.36±8.64	0.06±0.08	0.86±2.48
Others	24.73±22.90	10.80±9.32	13.01±22.05

LEfSe分析通过2组之间的比较,从而找到组间在丰度上有显著差异的特征或者生物标志物(物种),进一步显示了微生物组成的差异。如图5所示,与C组相比,葡萄球菌属 (LDA=5.50) 在M组中

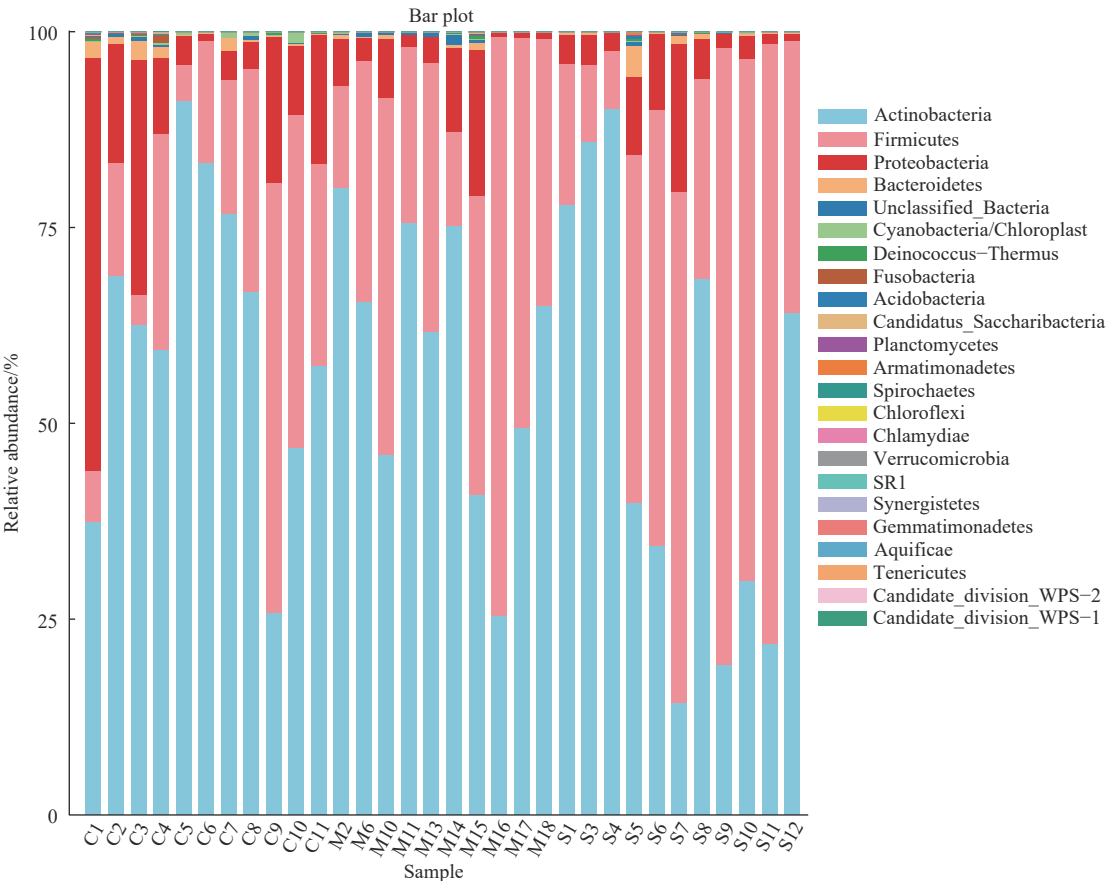


图 3 3 组人群毛囊菌群在门水平上相对丰度柱状图  
Fig 3 Column diagram of relative abundance of hair follicle microbiome at the phylum level in the three groups

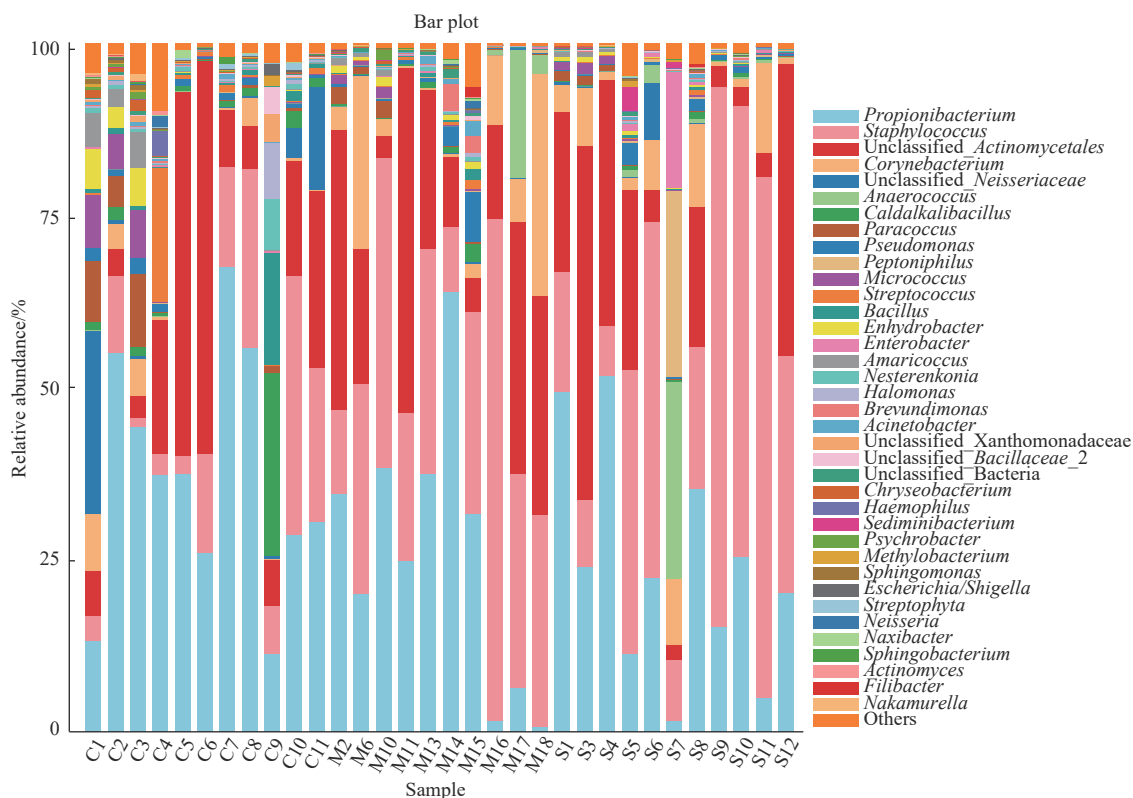
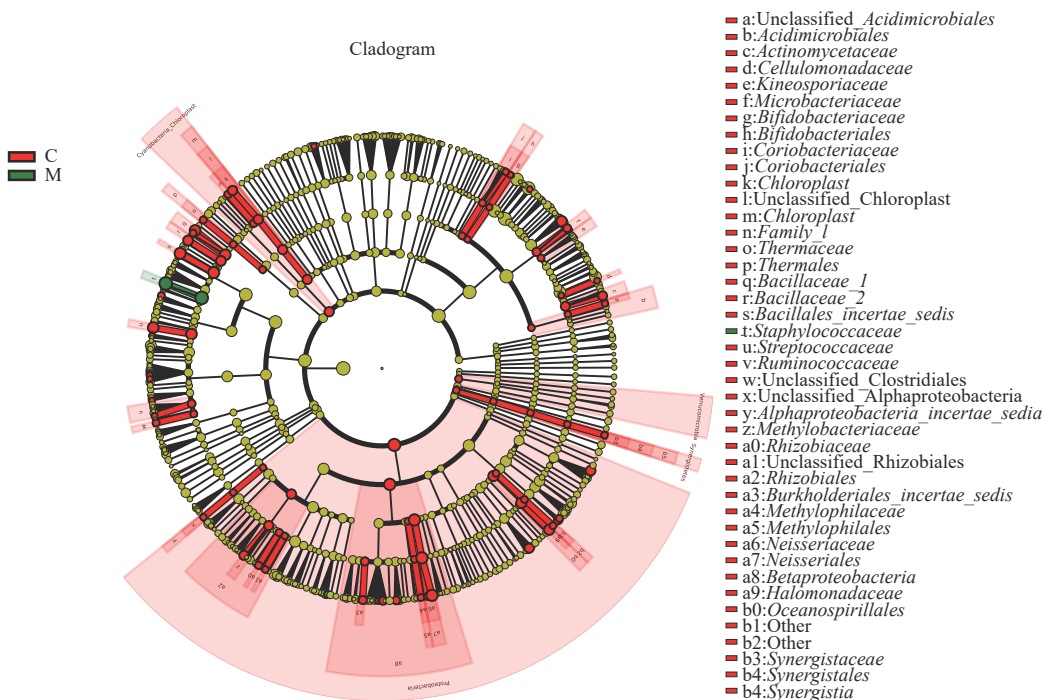


图4 3组人群毛囊菌群在属水平上相对丰度柱状图

Fig 4 Column diagram of relative abundance of hair follicle microbiome at the genus level in the three groups



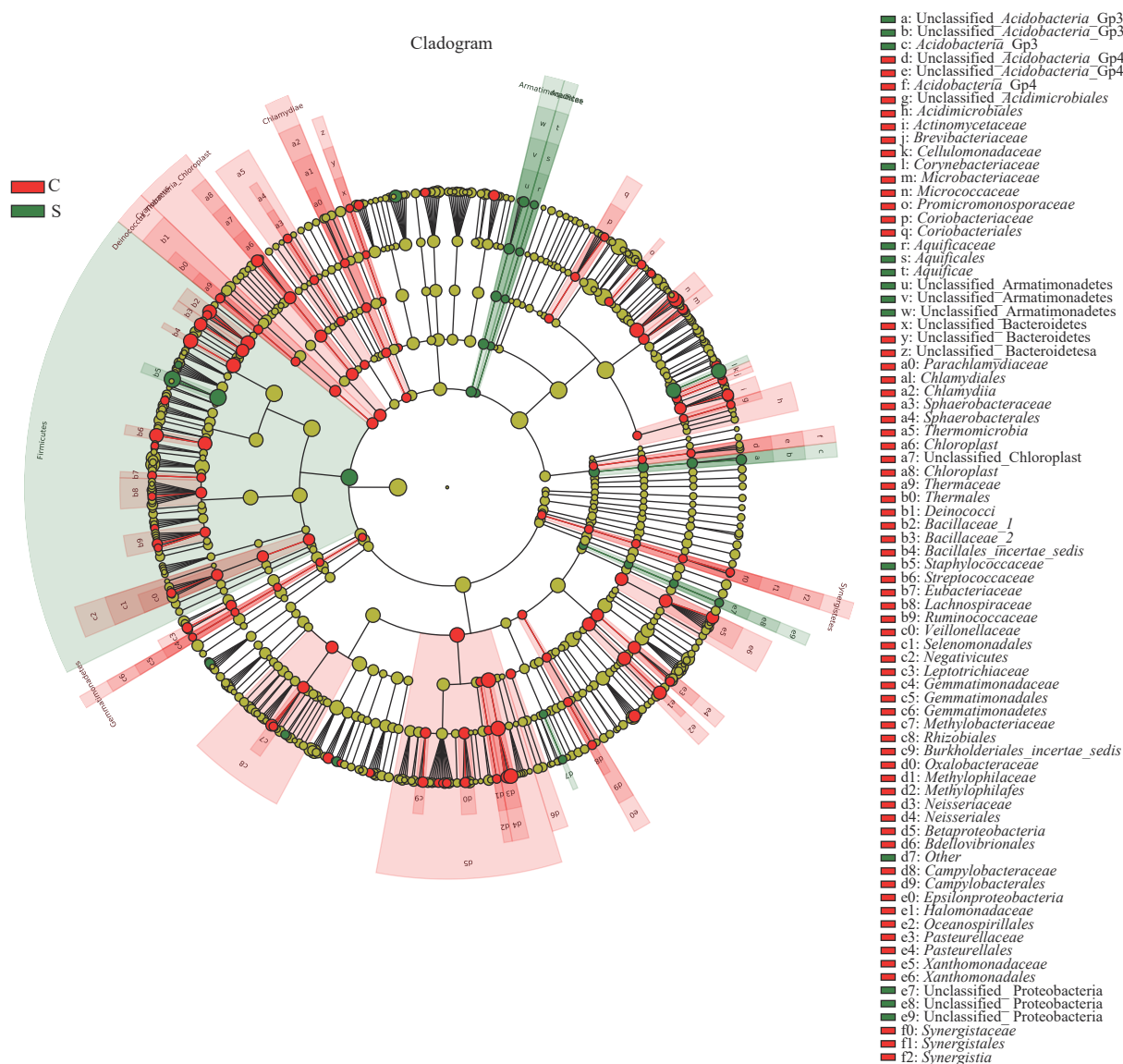
**Note:** The circles radiating from the inside out represent the classification levels from phylum to genus. Each circle and its shaded range at different classification levels represent the classification at that level, and the diameter of the circle is proportional to the relative abundance of the species. The species represented by the English letters next to the circles are shown in the legend. The red nodes represent microbial communities with high abundance and important roles in the red group, the green nodes represent microbial communities with important roles in the green group, and the yellow nodes represent species with no differences between groups.

图5 C组和M组人群毛囊菌群LEfSe分析进化分支图

Fig 5 LEfSe cladogram of analysis of follicular microbiome in C group and M group

起重要作用。如图6所示,与C组相比,葡萄球菌属 (LDA=5.57)、棒状杆菌属 (LDA=4.74)、沉积杆菌属 (*Sediminibacterium*, LDA=3.62)、Unclassified\_*Acidobacteria*\_Gp3 (LDA=2.66) 以及 *Gemmata*

(LDA=2.19) 在S组中起重要作用。如图7所示,与M组相比, Unclassified\_*Acidobacteria*\_Gp3 (LDA=2.66) 在S组中起重要作用。



**Note:** The circles radiating from the inside out represent the classification levels from phylum to genus. Each circle and its shaded range at different classification levels represent the classifications at those levels, and the diameter of the circle is proportional to the relative abundance of the species. The species represented by the English letters next to the circles are shown in the legend. The red nodes represent microbial communities with high abundance and important roles in the red group, the green nodes represent microbial communities with important roles in the green group, and the yellow nodes represent species with no differences between groups.

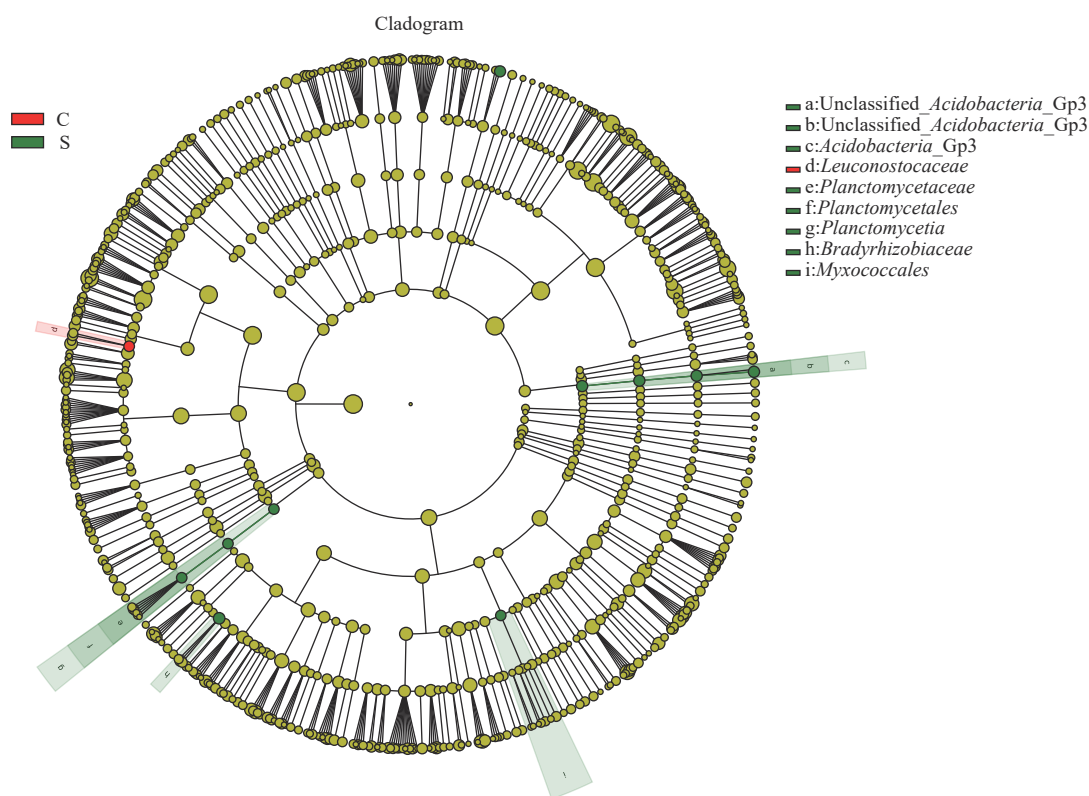
图6 C组和S组人群毛囊菌群LEfSe分析进化分支图

Fig 6 LEfSe cladogram of analysis of hair follicle microbiome in Group C and Group S

### 2.3 细菌绝对定量差异分析

qPCR结果如图8所示, C组细菌总拷贝数为 (17 262.28±11 439.72) 个/μL, M组和S组分别为 (67 506.34±38 265.74) 个/μL和 (188 674.75±

141 689.54) 个/μL。相对于C组, M组和S组样本中细菌拷贝数均显著增加 (均 $P=0.001$ ); S组毛囊样本中细菌载量显著高于M组, 差异有统计学意义 ( $P=0.017$ )。



**Note:** The circles radiating from the inside out represent the classification level from phylum to genus. Each circle and its shaded range at different classification levels represent the classification at that level, and the diameter of the circle is proportional to the relative abundance of the species. The species represented by the English letters next to the circle are shown in the legend. The red nodes represent microbial communities with high abundance and important roles in the red group, the green nodes represent microbial communities with important roles in the green group, and the yellow nodes represent species with no differences between groups.

图7 M组和S组人群毛囊菌群LEfSe分析进化分支图

Fig 7 LEfSe cladogram of analysis of hair follicle microbiome in Group M and Group S

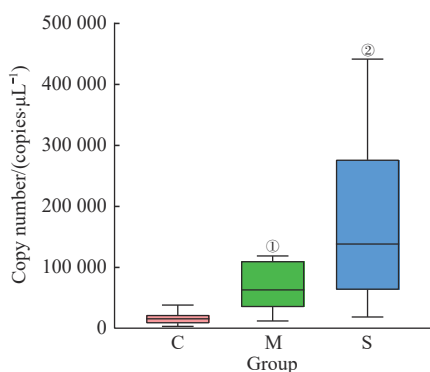


图8 3组人群毛囊细菌总拷贝数箱型图

Fig 8 Box plots of total number of follicular bacterial copies of the three groups

### 3 讨论

与肠道和其他身体组织一样,皮肤被密集的共生微生物群落定植,这些微生物群落占据着不同的皮肤环境生态位构成了皮肤微生态,其组成因宿主个体、身体部位、年龄和疾病状态而异<sup>[10]</sup>。皮肤微生物在抵御病原体入侵、调节免疫系统和分解天然产物方面

具有重要作用<sup>[11]</sup>。微生物群落之间及其与宿主之间广泛紧密的“对话”可以维持有效的皮肤功能。当这种平衡被打破时,可能导致痤疮、玫瑰痤疮、特应性皮炎等炎症性皮肤病的发生<sup>[12-13]</sup>。

本研究为明确中度痤疮、重度痤疮以及健康对照人群组间非皮损区毛囊菌群结构差异,对不同组别样本的毛囊菌群进行了 $\alpha$ 及 $\beta$ 多样性分析。 $\alpha$ 多样性分析发现,中度痤疮组和重度痤疮组相对于健康组菌群的Chao1指数差异有统计学意义,痤疮患者的毛囊菌群多样性较健康对照组明显降低,这一结论再次佐证了相对多样化的菌群是健康的微生物群这一观点<sup>[14]</sup>。基于Bray-Curtis距离的PCoA图表明健康组人群样本分布较为集中,组内差异较小;而中度痤疮组及重度痤疮组人群样本分布有一定的趋势但较为离散,组内之间存在较大差异;3组样本趋势分布差异明显,说明其微生物群落结构差异较大。相似性分析结果显示健康组与痤疮组相比 $\beta$ 多样性有显著差异,物种组成相似度低;2个痤疮组组间物种相似度较高。



本研究进一步对3组人群毛囊菌群的群落结构进行分析,发现在门水平上3组样本的优势细菌主要是由放线菌门、厚壁菌门、变形菌门、拟杆菌门组成,占比大于99%。相对于健康组人群,中度痤疮组毛囊中变形菌门相对丰度显著降低,重度痤疮组厚壁菌门的相对丰度显著升高。这提示痤疮患者毛囊菌群的多样性与痤疮的严重程度可能存在某种相关性。属水平上健康组优势菌为丙酸杆菌属、未分类的放线菌属,2个痤疮组优势菌为葡萄球菌属、丙酸杆菌属。随着痤疮严重程度增加,丙酸杆菌属相对丰度呈现下降趋势,但数据无统计学意义;相对于健康组,中度痤疮组和重度痤疮组非皮损区毛囊中的葡萄球菌属相对丰度均显著增加。

传统观点认为,痤疮的发生与痤疮丙酸杆菌(*Cutibacterium acnes*, *C. acnes*)的过度增殖有关,*C. acnes*能够分泌脂肪酶、透明质酸酶和蛋白酶,产生代谢物卟啉、短链脂肪酸等毒力因子,促进角质细胞增殖、诱导分化、炎症反应等诱发或加重痤疮的病情<sup>[15-16]</sup>。然而FITZ-GIBBON等<sup>[17]</sup>发现在健康者和痤疮患者的毛囊皮脂腺单位中,*C. acnes*均为最丰富的细菌,细菌丰度没有差异,而菌株种群结构显著不同。本研究进一步佐证了*C. acnes*并非痤疮发生的单一因素。此外,本研究通过LEfSe分析发现中度痤疮组和重度痤疮组中的葡萄球菌属均显著增加,提示其可能成为疾病发生的关键微生物。表皮葡萄球菌(*Staphylococcus epidermidis*, *S. epidermidis*)是毛囊皮脂腺单位中的另一代表性微生物,DRENO等<sup>[18]</sup>的研究表明在丘疹、脓疱皮损中*S. epidermidis*是最丰富的物种,但其在痤疮中具体作用及其机制尚不清晰。有研究发现其分泌的细菌素及代谢产物短链脂肪酸如乙酸、丁酸可以抑制*C. acnes*的定植和传播<sup>[19]</sup>;其病原相关分子模式脂磷酸刺激角质形成细胞表面Toll样受体2(Toll-like receptor 2, TLR2)产生miRNA-143,后者可以降低TLR2分子的表达数量,从而减少*C. acnes*通过TLR2产生的促炎因子<sup>[20]</sup>。表皮素(epidermin)为*S. epidermidis*分泌的羊毛硫抗生素家族主要成员之一,在极低浓度下便可作用于多种革兰阳性杆菌,通过与靶细胞壁上的脂质II结合使其穿孔导致细菌死亡<sup>[21]</sup>。*S. epidermidis*产生的电能还可以抑制*C. acnes*的生长以及造成细胞裂解<sup>[22]</sup>。另一方面,*S. epidermidis*可能作为致病菌参与痤疮的发生。DAGNELIE等<sup>[23]</sup>证实*S. epidermidis*似乎比

*C. acnes*在诱导先天免疫分子尤其是白细胞介素-6(interleukin-6, IL-6)、 $\gamma$ 干扰素诱导蛋白-10(interferon  $\gamma$ -inducible protein-10, IP-10)等方面具有更强的作用。

不同于轻度、中度痤疮,重度痤疮的临床表现以顽固性及难治性结节、囊肿,以及瘢痕为特征,并且对药物治疗效果不佳,病情反复发作迁延不愈。一项meta分析<sup>[24]</sup>显示我国重度痤疮占全部痤疮患者的比例为1.8%~6.7%。相比之下,重度痤疮对患者容貌及身心健康的影响更为严重。LI等<sup>[25]</sup>学者发现,4级痤疮患者的面部皮肤微生物群与1~3级痤疮患者相比差异显著,其 $\alpha$ 多样性更高,粪杆菌属、克雷伯菌属、臭杆菌属和拟杆菌属的比例增加。这一结论与本研究有所不符,推测这一现象可能与研究地域、样本选择以及取样时间等多种因素有关。

本研究对3组样本进行qPCR定量分析发现,随着痤疮严重程度的增加,毛囊内细菌载量显著增加。已往的研究<sup>[26-28]</sup>发现,在肠道系统中出现的小肠细菌过度增长可能与肠易激综合征、慢性胃病、糜烂性食管炎等多种疾病相关。具体的机制可能为小肠细菌过度生长引起细菌产物改变,如维持肠道屏障的细菌产物减少导致肠道通透性改变;小肠细菌过度生长导致肠道内炎症反应等<sup>[29]</sup>。考虑到毛囊皮脂腺单位与肠道在一定程度上具有相似的结构与功能,我们推测当外界环境或内在因素发生变化时,改变了毛囊皮脂腺单位中稳定的环境,宿主免疫反应失调,引起菌群失调、致病菌过度繁殖,并形成负反馈,可能在一定程度上导致了痤疮疾病的发生发展。

综上所述,本研究结合16S rRNA测序以及qPCR探索了不同严重程度痤疮患者非皮损区毛囊菌群多样性、物种组成和载量的差异,表明在痤疮患者的最早期皮损(微粉刺)中微生态已经发生了改变。痤疮的发生及严重程度可能与毛囊菌群变化存在着密切的联系。这一发现可能为深入探究微生物介导的痤疮恶化机制,以及开发基于微生物的痤疮治疗方法提供有力的证据。基于本研究中筛选出的特异性菌群标志物,后期将扩大样本量、结合多组学分析和体内动物实验等进一步明确其是否参与疾病发生发展及相关作用机制。

#### 利益冲突声明/Conflict of Interests

所有作者声明不存在利益冲突。

All authors disclose no relevant conflict of interests.

### 伦理批准和知情同意/Ethics Approval and Patient Consent

本研究涉及的所有实验均已通过上海交通大学医学院附属仁济医学伦理委员会的审核批准(文件号KY2022-041-B)。所有受试者均签署书面知情同意书。

All experimental protocols in this study were reviewed and approved by the Medical Ethics Committee of Renji Hospital, Shanghai Jiao Tong University School of Medicine (Approval Letter No. KY2022-041-B), and consent letters have been signed by people in the research.

### 作者贡献/Authors' Contributions

梁梦晨负责研究的构思、设计和实施;梁梦晨、李嘉祺、莫小辉负责论文撰写与修改;梁梦晨、李嘉祺负责菌群检测和统计分析;

梁梦晨、吴心怡负责生物样本的采集;鞠强参与论文审阅与修改。所有作者均阅读并同意了最终稿件的提交。

The study was conceived, designed, and implemented by LIANG Mengchen. The manuscript was drafted and revised by LIANG Mengchen, LI Jiaqi and MO Xiaohui. The microbiota analysis and statistical analysis were performed by LIANG Mengchen and LI Jiaqi. The biosamples were collected by LIANG Mengchen and WU Xinyi. JU Qiang reviewed and revised the manuscript. All the authors have read the last version of paper and consented for submission.

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## 参 · 考 · 文 · 献

- [1] CHEN Y, KNIGHT R, GALLO R L. Evolving approaches to profiling the microbiome in skin disease[J]. *Front Immunol*, 2023, 14: 1151527.
- [2] LAYTON A M, RAVENSCROFT J. Adolescent acne *Vulgaris*: current and emerging treatments[J]. *Lancet Child Adolesc Health*, 2023, 7(2): 136-144.
- [3] BERRY K, LIM J, ZAENGLEIN A L. Acne *Vulgaris*: treatment made easy for the primary care physician[J]. *Pediatr Ann*, 2020, 49(3): e109-e115.
- [4] CHUA W, POH S E, LI H. Secretory proteases of the human skin microbiome[J]. *Infect Immun*, 2022, 90(1): e0039721.
- [5] SHI J, CHENG J W, ZHANG Q, et al. Comparison of the skin microbiota of patients with acne *Vulgaris* and healthy controls[J]. *Ann Palliat Med*, 2021, 10(7): 7933-7941.
- [6] NUMATA S, AKAMATSU H, AKAZA N, et al. Analysis of facial skin-resident microbiota in Japanese acne patients[J]. *Dermatology*, 2014, 228(1): 86-92.
- [7] XU X X, RAN X, TANG J Q, et al. Skin microbiota in non-inflammatory and inflammatory lesions of acne *Vulgaris*: the underlying changes within the pilosebaceous unit[J]. *Mycopathologia*, 2021, 186(6): 863-869.
- [8] WITKOWSKI J A, PARISH L C. The assessment of acne: an evaluation of grading and lesion counting in the measurement of acne[J]. *Clin Dermatol*, 2004, 22(5): 394-397.
- [9] SPESHOCK J L, BRADY J A, EASTMAN J, et al. Impact of manure storage time and temperature on microbial composition and stable fly (Diptera: Muscidae) development[J]. *AiM*, 2019, 9(3): 248-265.
- [10] LEE Y B, BYUN E J, KIM H S. Potential role of the microbiome in acne: a comprehensive review[J]. *J Clin Med*, 2019, 8(7): 987.
- [11] BYRD A L, BELKAID Y, SEGRE J A. The human skin microbiome[J]. *Nat Rev Microbiol*, 2018, 16(3): 143-155.
- [12] KOH L F, ONG R Y, COMMON J E. Skin microbiome of atopic dermatitis[J]. *Allergol Int*, 2022, 71(1): 31-39.
- [13] SUNG K H. Microbiota in *Rosacea*[J]. *Am J Clin Dermatol*, 2020, 21(Suppl 1): 1-11.
- [14] SKOWRON K, BAUZA-KASZEWSKA J, KRASZEWSKA Z, et al. Human skin microbiome: impact of intrinsic and extrinsic factors on skin microbiota[J]. *Microorganisms*, 2021, 9(3): 543.
- [15] ISARD O, KNOL A C, ARIÈS M F, et al. *Propionibacterium acnes* activates the IGF-1/IGF-1R system in the epidermis and induces keratinocyte proliferation[J]. *J Invest Dermatol*, 2011, 131(1): 59-66.
- [16] AKAZA N, AKAMATSU H, KISHI M, et al. Effects of *Propionibacterium acnes* on various mRNA expression levels in normal human epidermal keratinocytes *in vitro*[J]. *J Dermatol*, 2009, 36(4): 213-223.
- [17] FITZ-GIBBON S, TOMIDA S, CHIU B H, et al. *Propionibacterium acnes* strain populations in the human skin microbiome associated with acne[J]. *J Invest Dermatol*, 2013, 133(9): 2152-2160.
- [18] DRENO B, MARTIN R, MOYAL D, et al. Skin microbiome and acne *Vulgaris*: *Staphylococcus*, a new actor in acne[J]. *Exp Dermatol*, 2017, 26(9): 798-803.
- [19] O'NEILL A M, NAKATSUJI T, HAYACHI A, et al. Identification of a human skin commensal bacterium that selectively kills cutibacterium *acnes*[J]. *J Invest Dermatol*, 2020, 140(8): 1619-1628. e2.
- [20] XIA X L, LI Z H, LIU K W, et al. Staphylococcal LTA-induced miR-143 inhibits *Propionibacterium acnes*-mediated inflammatory response in skin[J]. *J Invest Dermatol*, 2016, 136(3): 621-630.
- [21] COTTER P D, HILL C, ROSS R P. Bacterial lantibiotics: strategies to improve therapeutic potential[J]. *Curr Protein Pept Sci*, 2005, 6(1): 61-75.
- [22] MARITO S, KESHARI S, TRAISAENG S, et al. Electricity-producing *Staphylococcus epidermidis* counteracts *Cutibacterium acnes*[J]. *Sci Rep*, 2021, 11(1): 12001.
- [23] DAGNELIE M A, CORVEC S, TIMON-DAVID E, et al. *Cutibacterium acnes* and *Staphylococcus epidermidis*: the unmissable modulators of skin inflammatory response[J]. *Exp Dermatol*, 2022, 31(3): 406-412.
- [24] LI D H, CHEN Q, LIU Y, et al. The prevalence of acne in Mainland China: a systematic review and meta-analysis[J]. *BMJ Open*, 2017, 7(4): e015354.
- [25] LI C X, YOU Z X, LIN Y X, et al. Skin microbiome differences relate to the grade of acne *Vulgaris*[J]. *J Dermatol*, 2019, 46(9): 787-790.
- [26] KÖLJALG U, NILSSON R H, ABARENKOV K, et al. Towards a unified paradigm for sequence-based identification of fungi[J]. *Mol Ecol*, 2013, 22(21): 5271-5277.
- [27] SU T T, LAI S C, LEE A, et al. Meta-analysis: proton pump inhibitors moderately increase the risk of small intestinal bacterial overgrowth[J]. *J Gastroenterol*, 2018, 53(1): 27-36.
- [28] PIMENTEL M, SAAD R J, LONG M D, et al. ACG clinical guideline: small intestinal bacterial overgrowth[J]. *Am J Gastroenterol*, 2020, 115(2): 165-178.
- [29] EFREMOVA I, MASLENNIKOV R, POLUEKTOVA E, et al. Epidemiology of small intestinal bacterial overgrowth[J]. *World J Gastroenterol*, 2023, 29(22): 3400-3421.

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