Subgingival Microbial Profiles of Young Chinese Adults with Stage I/II Periodontitis, Gingivitis and Periodontal Health Status

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Objective: *To analyse the subgingival microbiota of Stage I/II periodontitis, gingivitis with different degrees of severity, and periodontal health in subjects in a Chinese young adult population.*

Methods: *Subgingival plaque samples were collected from 15 Stage I/II periodontitis patients, 38 gingivitis patients and 15 periodontally healthy individuals, all aged from 18 to 21 years. Gingivitis patients were divided into two subgroups according to the Bleeding Index (BI) of their sampled teeth: gingivitis with above median BI (G-HBI) and below median BI (G-LBI). The subgingival plaque samples were collected from teeth 16, 26, 36, 46, 11 and 31 according to FDI notation. The V3-V4 region of the 16S rRNA gene of all the samples was sequenced and analysed.*

Results: *The Stage I/II periodontitis, gingivitis and periodontal health groups showed distinct subgingival microbiota profiles. When the gingivitis patients were stratified into two subgroups, the community structure of G-HBI showed no significant difference from early-stage periodontitis, but differed from G-LBI and the healthy group. Most periodontitis-related taxa were most abundant in Stage I/II periodontitis, followed by G-HBI, G-LBI and the periodontally healthy group. Porphyromonas gingivalis, Filifactor alocis, Tannerella forsythia, Saccharibacteria TM7 G-5 356, Lachnospiraceae G-8 500, Peptostreptococcaceae spp. and Syntrophomonadaceae VIIIG-1 435 were associated with Stage I/II periodontitis. Porphyromonas 275, Leptotrichia 417 and Saccharibacteria TM7 G-2 350 were associated with gingivitis. Porphyromonas gingivalis was significantly more abundant in G-HBI than in G-LBI.*

Conclusion: *Within the limitations of this preliminary study, gingivitis and early-stage periodontitis were associated with an increased degree of dysbiosis in the subgingival microbiota in a Chinese young adult population.*

Key words: *gingivitis, microbiota, periodontitis, sequencing, young adults*
Chin J Dent Res 2021;24(3):167–175; doi: 10.3290/j.cjdr.b1965003

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Plaque accumulation is the initial cause of periodontal infection[1]. Persistent gingival inflammation is regarded as a risk factor for the development of periodontitis[2], and subgingival plaque is crucial in this process[3]. Numerous studies have investigated the subgingival microbiota using various techniques ranging from bacteria culture to polymerase chain reaction (PCR), DNA-DNA hybridisation, gene probes and high-throughput sequencing. Earlier studies using targeted techniques have demonstrated that periodontal disease is associated with the change of bacteria composition[4], and some periodontal pathogens have been well established, namely *Tannerella forsythia* (*T. forsythia*), *Porphyromonas gingivalis* (*P. gingivalis*) and *Treponema denticola*, known as "red complex"[3]. The function and virulence factors of these bacteria have been intensively studied both *in vitro* and in animal models[5].

It has been estimated that there are approximately 700 species of bacteria in the human oral microbiome[6]. Within the limitations of targeted techniques, only a subset of microbes could be detected. Recently, the advent of next-generation sequencing (NGS) has enabled a global view of the oral microbiome. Multiple studies have investigated the subgingival bacterial community under various periodontal conditions in different populations and revealed that the shift of the subgingival microbiota from health to disease is characterised by increased bacterial biomass, alteration of diversity and the change of composition from symbiosis to dysbiosis[7-19]. Although certain periodontitis-associated microorganisms may contribute more effectively to the shift of the subgingival community, the whole microbial community rather than an individual member plays a more important role in the pathogenesis of periodontal disease[20]. However, most previous studies using NGS have focused on the comparison of the subgingival microbiota between health and moderate to severe periodontitis[8-10,12,13,15-17]. Fewer studies have examined gingivitis[7,10,11,13,14,21] and early-stage periodontitis[18,19]. Moreover, comparisons of gingivitis and early-stage periodontitis are scarce[22]. In addition, data from previous studies on gingivitis using NGS have revealed that some of the members of the core microbiota in gingivitis show an overlap with both health and periodontitis[10,13,14], indicating that the microbial community of gingivitis may represent a transition state between health and periodontitis. However, some other gingivitis-enriched taxa were not strongly associated with periodontitis or health, suggesting that the profile of the gingivitis community structure might be somewhat unique[7,10,11,13,23]. The different degrees of severity of gingivitis may be a confounding factor in interpreting these results, and there has been less evidence of the association of the subgingival microbiota with different degrees of severity of gingivitis[10,11].

It is therefore particularly important to investigate the subgingival microbiota in health, gingivitis and early-stage periodontitis. In addition, microbiota of gingivitis of different degrees of severity need to be compared with periodontitis and health separately. The present study therefore aimed to analyse the subgingival microbial community in health, gingivitis of different degrees of severity and early-stage periodontitis in a young adult Chinese population.

Materials and methods

This was a cross-sectional study. The participants were all recruited from Cangzhou Technical College, Cangzhou,

Hebei Province, China, and provided written informed consent. The examination and sample collection were completed at a dental clinic in Cangzhou, with approval from the Ethics Committee of Peking University School and Hospital of Stomatology (PKUSSIRB-201944054).

The participants were young adults aged 18 to 21 years. The inclusion criteria for Stage I/II periodontitis patients were as follows:
- diagnosis of periodontitis according to the new classification scheme for periodontal diseases and conditions introduced in 2018[24];
- maximum interdental clinical attachment loss (CAL) ≤ 4 mm and maximum probing depth (PD) ≤ 5 mm[24].

The inclusion criteria for the gingivitis group were as follows:
- intact periodontium;
- full-mouth bleeding on probing (BOP) score ≥ 10%;
- no CAL[25].

The inclusion criteria for healthy individuals were as follows:
- full-mouth PD ≤ 3 mm;
- full-mouth BOP < 10%;
- no CAL[25].

The exclusion criteria were as follows:
- fewer than 20 natural teeth;
- smoking;
- systemic disease;
- presence of orthodontic appliances;
- presence of caries or prostheses in sampled teeth;
- patients having received periodontal treatment within the previous 6 months;
- use of antibiotic, anti-inflammatory or anticoagulant therapy within the previous 3 months;
- self-reported pregnancy or lactation.

After a brief screening, the enrolled participants received a full-mouth periodontal examination, including full-mouth PD, CAL, BOP and PD of the sampled teeth, Bleeding Index (BI)[26] and Plaque Index (PI)[27]. All the examinations and sample collections were conducted by a single calibrated periodontist. To examine the interexaminer reproducibility of measurements, 10 participants were randomly selected and reexamined. The kappa values for PD and BI were 0.89 and 0.81, respectively. A total of 15 periodontitis patients, 38 gingivitis patients and 15 periodontally healthy individuals aged 18 to 21 years were enrolled in the study.

The subgingival plaque was collected 1 week after the clinical examination. The samples were collected
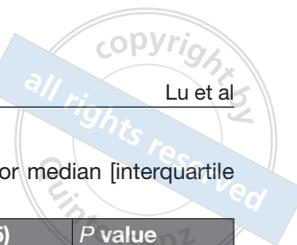
Table 1 Demographic information and clinical variables for sampled teeth (mean ± standard deviation or median [interquartile range]) for periodontitis, gingivitis and health groups.

Variable		Periodontitis (n = 15)	G-HBI (n = 19)	G-LBI (n = 19)	Health (n = 15)	P value
Age (y)		19.0 [0.0]	19.0 [1.0]	19.0 [1.5]	19.2 ± 0.9	0.696*
Sex (% female)		80.0	63.2	57.9	60.0	0.554†
Clinical variables for sampled teeth	PI	2.2 ± 0.4‡	1.8 ± 0.3‡	1.7 (0.7)§	1.4 ± 0.4§	< 0.001*
	BI	2.5 ± 0.7‡	1.8 [0.4] ‡ǁ	1.4 [0.2]‡§	0.3 [0.2]§	< 0.001*
	PD (mm)	2.5 ± 0.2‡	2.3 ± 0.2§	2.2 ± 0.2‡§	1.9 ± 0.2§	< 0.001¶

*Kruskal-Wallis test, and the post hoc test was a Nemenyi test. †Chi-square test. ‡Significantly different compared with health group, $P < 0.05$. §Significantly different compared with periodontitis group, $P < 0.05$. ǁSignificantly different between G-HBI and G-LBI, $P < 0.05$. ¶ANOVA test, and the post hoc test was a Tukey test.

from the mesiobuccal and mesiolingual sites of teeth 16, 26, 36, 46, 11 and 31. After isolation of the sampled teeth from saliva and removal of supragingival plaque, sterile Gracey curettes (Hu-Friedy, Chicago, IL, USA) were inserted in the subgingival area to collect the samples. The 12 samples from each subject were pooled and transferred into a sterile Eppendorf tube (Eppendorf, Hamburg, Germany) and stored at −80°C until being used.

DNA was extracted from the subgingival plaque samples using MoBio PowerSoil DNA Isolation Kit (100) (MoBio Laboratories, Carlsbad, CA, USA) according to the manufacturer's protocol. The extracted DNA was amplified via PCR using primers targeting the V3-V4 regions of the bacterial 16S rRNA gene. The PCR products were purified using AMPure beads (Beckman Coulter, Brea, CA, USA) and quantified using quantitative PCR (qPCR), then sequenced on the MiSeq PE300 (Illumina, San Diego, CA, USA) at Allwegene Technology (Beijing, China).

The raw sequencing data were quality-filtered, and short sequences, primers and chimeric sequences were removed to obtain high-quality sequences. The clean tags of all the samples were clustered into operational taxonomic units (OTUs) using QIIME (version 1.8.0)[28] at 97% sequence identity by UPARSE[29]. Taxonomies were assigned using the Ribosomal Database Project (RDP) Classifier (Michigan State University, East Lansing, MI, USA) based on the SILVA (release 128, http://www.arb-silva.de) 16S rRNA database[30].

For each subject, the BI score was calculated as the mean BI of the sampled teeth. Gingivitis patients were divided into two subgroups according to the median BI score. Individuals with a BI score above the median (51st to 100th percentile) were categorised as gingivitis with higher BI (G-HBI), and those with a below median BI score (0 to 50th percentile) were categorised as gingivitis with lower BI (G-LBI). The clinical parameters and alpha diversity among the periodontitis,

G-HBI, G-LBI and health groups were assessed using an analysis of variance (ANOVA) or Kruskal-Wallis test. Analysis of beta-diversity utilised principal coordinate analysis (PCoA) of the Bray-Curtis distance based on OTUs using the R packages vegan (version 2.5-6) and ape (version 5.3) (both R Core Team, Vienna, Austria). Permutational multivariate analysis of variance (PERMANOVA) was used to test significance across the groups. The multiple testing *P* values were corrected using the false discovery rate (FDR) method. For differential abundance comparisons among groups, analysis of composition of microbiomes (ANCOM, version 3.6.2, R Core Team) was used. ANCOM was based on log ratios, accounting for compositionality of microbiome data, and greatly controlled the FDR while maintaining high statistical power. The results for the 0.7 detection level were considered significant, and a medium stringent multiple comparison correction option (multicorr = 2) was chosen[31,32]. Since the ANCOM results only provided a list of taxa that differed in composition, bar charts were plotted for mean relative abundance to show the abundance of taxa that varied in composition among the groups.

Results

The participants included 15 periodontitis patients, 38 gingivitis patients and 15 periodontally healthy subjects aged 18 to 21 years. The two gingivitis subgroups (G-HBI and G-LBI) had 19 participants. The demographic information and clinical variables for the sampled teeth are presented in Table 1. The age and sex of the participants in the four groups did not differ significantly.

For each periodontitis patient, the maximum CAL was ≤ 2 mm, and at least one tooth with CAL, BOP and PD ≥ 4 mm was sampled. All the clinical parameters showed the highest mean value in the periodontitis group, followed by the G-HBI, G-LBI and health groups. A statistically significant difference was
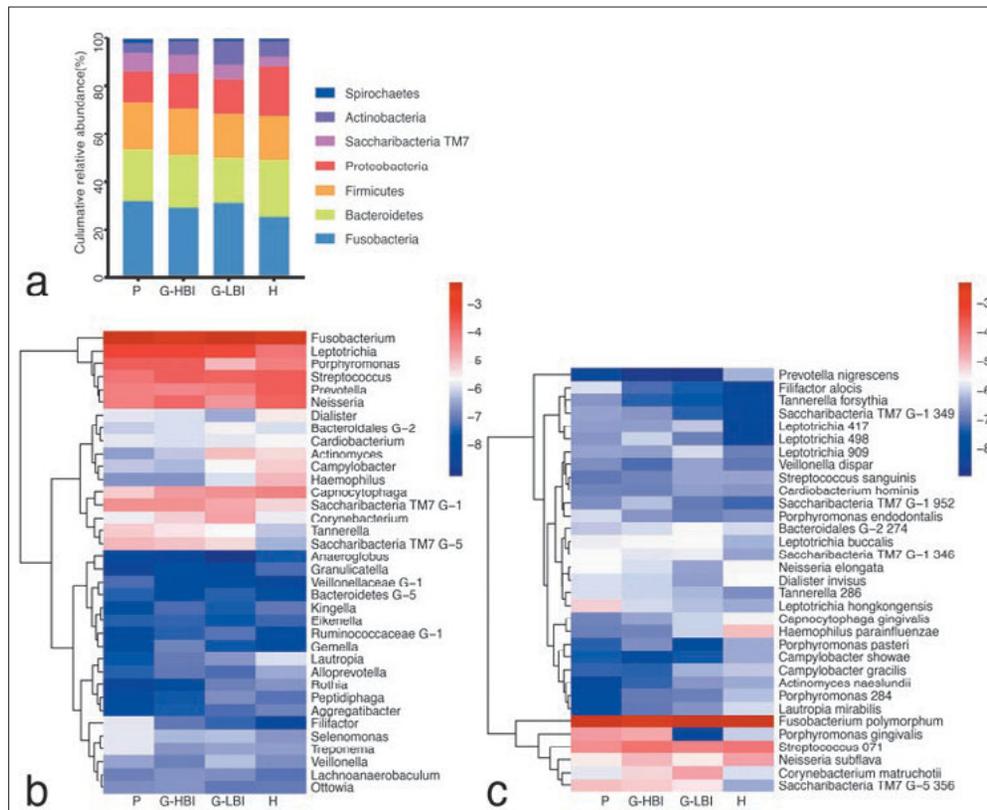
Fig 1 Composition of subgingival microbiota in each group. **(a)** Phylum level. The seven most abundant phyla are shown. **(b)** Genus level. Genera with mean relative abundance ≥ 0.5% in at least one group are shown. **(c)** Species level. Species with mean relative abundance ≥ 1% in at least one group are shown. The relative abundances in **(b)** and **(c)** were log2 transformed. G-HBI, gingivitis group with above median BI; G-LBI, gingivitis group with below median BI; H, health group; P, periodontitis group.

observed for all clinical parameters between periodontitis and health, G-HBI and health, and between periodontitis and G-LBI. Patients with periodontitis showed deeper PD in sampled teeth than those in the G-HBI group. PI was not significantly different between the G-HBI and G-LBI groups, while G-HBI patients exhibited higher BI than G-LBI patients.

A total of 4,546,228 high-quality sequences were obtained, with a mean of 66,856 sequences per sample (ranging from 25,443 to 248,933). Clustering of all high-quality sequences at 97% identity resulted in 3570 OTUs (275 to 1105 OTUs per sample, mean 490 ± 179). In total, 13 phyla, 28 classes, 50 orders, 95 families, 202 genera and 574 species were detected in the subgingival microbiota.

The most abundant phyla of all the samples were *Fusobacteria*, *Bacteroidetes*, *Firmicutes*, *Proteobacteria*, *Saccharibacteria* TM7, *Actinobacteria* and *Spirochaetes*, which accounted for > 98% of all the bacteria (Fig 1a). The distribution of genera with > 0.5% mean relative abundance in at least one group is shown in Fig 1b. Fusobacterium was the most predominant component with a mean relative abundance greater than 20%, followed by subdominant genus of *Leptotrichia*, *Streptococcus*, *Neisseria*, *Porphyromonas*

and *Prevotella*, all of which showed mean abundance > 5%. At species level, the five most abundant species were *Fusobacterium polymorphum*, *Streptococcus* 071, *Neisseria subflava*, *Corynebacterium matruchotii* and *Saccharibacteria* TM7 G-5 356, which accounted for 32.8% of the whole sample (Fig 1c).

Alpha diversity measured using the Shannon index is shown in Fig 2a. G-LBI had the highest Shannon index, followed by G-HBI, periodontitis and the healthy group, but no statistical significance was observed among the four groups ($P = 0.081$). When G-LBI and G-HBI were combined as a whole gingivitis group, the Shannon index was significantly higher than in the healthy group ($P = 0.026$).

Regarding beta diversity, the community structure of periodontitis, gingivitis and health was significantly distinct (Fig 2b). G-HBI displayed a significantly different community structure compared with G-LBI ($P = 0.028$) (Fig 2c). When comparing G-HBI and G-LBI with periodontitis and health separately, the two gingivitis groups showed different results. In G-HBI, the community structure showed no significant difference with periodontitis ($P = 0.052$) but differed significantly from health ($P = 0.006$) (Fig 2d); however, the community structure of G-LBI was significantly
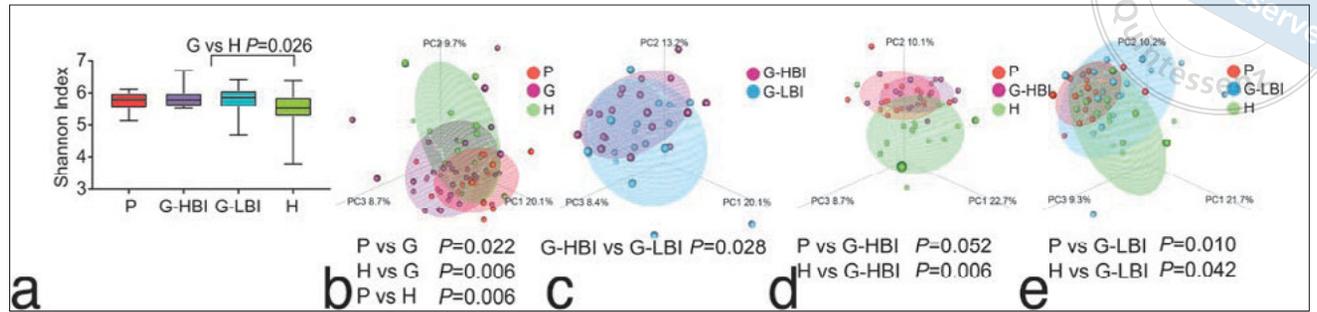
Fig 2 Alpha and beta diversity. **(a)** Shannon index of periodontitis, G-HBI, G-LBI and health. To compare gingivitis, health and periodontitis, communities from G-HBI and G-LBI were combined. *P* values were obtained with a Nemenyi test. **(b)** to **(e)** Beta diversity of periodontitis, G-HBI, G-LBI and health assessed by PCoA plots. **(b)** Samples in periodontitis, gingivitis and health groups. **(c)** Samples in G-HBI and G-LBI groups. **(d)** Samples in periodontitis, G-HBI and health groups. **(e)** Samples in periodontitis, G-LBI and health groups. *P* values were obtained with a PERMANOVA test with FDR correction.
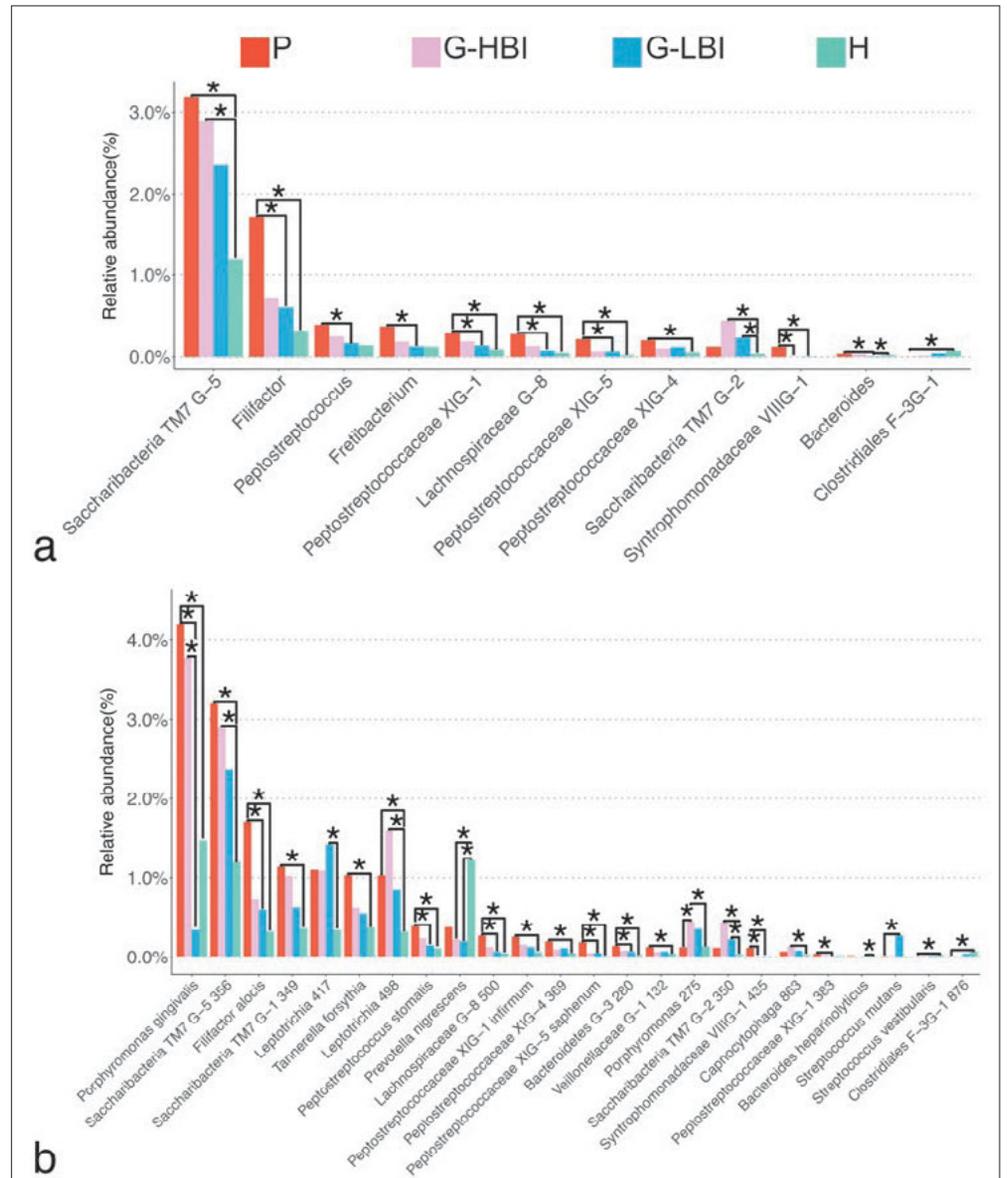

Fig 3 Taxa with differential abundance among the periodontitis, G-HBI, G-LBI and health groups. **(a)** Genera with significant difference in abundance among the four groups (ANCOM). **(b)** Species with significant difference in abundance among the four groups (ANCOM). *, Significant between groups (ANCOM).

different from both periodontitis ($P = 0.010$) and health ($P = 0.042$) (Fig 2e).

The differential abundance of taxa among the four groups at phylum, genus and species level was analysed by ANCOM. At phylum level, no significant difference in taxa abundance was detected among the four groups. The differential abundance of taxa at genus and species level are shown in Figs 3a and b, respectively.

At genus level, 12 genera were detected with significantly different abundance across the four groups (Fig 3a). Six genera were significantly enriched in periodontitis compared to in health, including *Saccharibacteria* TM7 G-5, *Filifactor*, *Lachnospiraceae* G-8, *Peptostreptococcaceae* XIG-1, XIG-4 and XIG-5. The low-abundance genus *Clostridiales* F-3G-1 was more abundant in health than in periodontitis. Between G-HBI and periodontitis, only *Syntrophomonadaceae* VIIIG-1 showed differential abundance, whereas eight genera exhibited significantly different abundance between G-LBI and periodontitis. When G-HBI and G-LBI were compared, no genus was detected with significantly different abundance. The abundance of *Saccharibacteria* TM7 G-5 and *Saccharibacteria* TM7 G-2 was greater in G-HBI than in health, while in G-LBI, only *Saccharibacteria* TM7 G-2 was more abundant than in health.

At the species level, 24 species were detected with significantly different abundance among the four groups (Fig 3b). The abundance of 15 species differed significantly between periodontitis and health. Of the 15 species, 13 were enriched in the periodontitis group, including *P. gingivalis*, *Filifactor alocis* (*F. alocis*) and *T. forsythia*, and two were more abundant in the healthy group. Between G-HBI and periodontitis, there were only two differentially abundant species, *Syntrophomonadaceae* VIIIG-1 435 and *Porphyromonas* 275. The abundance of *Syntrophomonadaceae* VIIIG-1 435 was greater in periodontitis, while *Porphyromonas* 275 was more abundant in G-HBI. However, between G-LBI and periodontitis, eight species showed significantly higher abundance in periodontitis. When comparing G-HBI and the healthy group, five species showed greater abundance in G-HBI, including *Saccharibacteria* TM7 G-5 356, *Leptotrichia* 498, *Porphyromonas* 275, *Saccharibacteria* TM7 G-2 350 and *Capnocytophaga* 863. As for G-LBI, only *Leptotrichia* 417 and *Saccharibacteria* TM7 G-2 350 showed greater abundance than in the healthy group. In health, *Prevotella nigrescens* (*P. nigrescens*) was significantly more abundant than G-HBI and G-LBI. Between G-HBI and G-LBI, only *P. gingivalis* showed substantially higher abundance in G-HBI; no other differential abundant taxa were detected.

Discussion

Most previous studies have focused on the subgingival microbiome in moderate to severe periodontitis compared with health. Research on the subgingival microbiome from health and gingivitis to early-stage periodontitis is scarce. In the present study, the subgingival microbiota in Stage I/II periodontitis, mild gingivitis, severe gingivitis and periodontal health in young adults were investigated by 16S rRNA gene sequencing. To our knowledge, the present study is the first to report the subgingival microbiota results from four such groups using high-throughput sequencing. Our findings showed that even at a very early stage, the subgingival bacterial community of periodontitis revealed a profile distinct from gingivitis and health. When gingivitis patients were stratified into two subgroups based on BI and compared with early-stage periodontitis and health separately, the community structure of G-HBI showed no significant difference from periodontitis, but differed from the G-LBI and health groups, indicating a more similar subgingival microbial profile between severe gingivitis and periodontitis. Most periodontitis-related taxa showed the highest abundance in Stage I/II periodontitis, followed by G-HBI, G-LBI and health. The present study provided a comprehensive picture of the subgingival microbiota from health and gingivitis to early-stage periodontitis. Although our study was cross-sectional, the data may represent different stages of dysbiosis from health to initial periodontal destruction, which may help to better understand the aetiology of initial periodontitis. The results also implied that severe gingivitis may increase susceptibility to periodontitis, and should be paid more attention in clinical practice (for example, through more frequent recall appointments).

In the present study, the periodontitis patients were different from those in most previous studies applying a NGS approach. In previous studies, periodontitis cases were more severe[8-10,13,15]. All the periodontitis cases in the present study were in early adulthood and showed maximum CAL ≤ 2 mm and PD ≤ 5 mm, demonstrating an initial stage of periodontitis. Even at this early stage, their subgingival community structure still showed a profile distinct from gingivitis and health. This finding was supported by a recent study on the subgingival microbiome in an elderly cohort, in which the mild periodontitis and health groups also exhibited different community structures[19]. In our data, we found 13 species with significantly higher abundance in periodontitis than in health. *P. gingivalis* and *T. forsythia* were among these species. They were also reported with increased abundance in initial periodontitis in earlier

studies using a non-NGS approach[22,33]. Other species in our data, such as *F. alocis*, *Saccharibacteria* TM7 G-5 356, *Saccharibacteria* TM7 G-1 349, *Leptotrichia* 498, *Lachnospiraceae* G-8 500 and *Peptostreptococcaceae spp.*, were reported to be associated with moderate to severe periodontitis and with increased PD in previous studies[8-10,13,15,19]. Interestingly, we found them to be enriched in this young cohort with very early-stage periodontitis, indicating that these species had already been implicated in the initial process associated with periodontal destruction.

Between severe gingivitis (G-HBI) and periodontitis, *Syntrophomonadaceae* VIIIG-1 435 was the only differential taxon with significantly higher abundance in periodontitis. Although its abundance was very low, it was present in 11 out of 15 subjects in the periodontitis group, while in other groups, it was detected in only two subjects. *Syntrophomonadaceae*-affiliated species were reported to be active in syntrophic butyrate degradation in anaerobic natural environments, such as paddy field soil[34] and eutrophic lake sediment[35], but it was rarely reported in human oral microbiota. Gomes et al[36] found it to be prevalent in deep pockets from combined periodontic-endodontic lesions. We inferred that the presence of *Syntrophomonadaceae* VIIIG-1 might be associated with the higher levels of butyrate in periodontitis sites produced by periodontal pathogens[37].

In our study, the gingivitis patients were stratified into two subgroups according to BI, and their subgingival microbiota were first compared separately with the periodontitis and health groups. After stratification, the results of alpha and beta diversity and differential abundant taxa all suggested that the subgingival community signature of G-HBI seemed more similar to early-stage periodontitis, while G-LBI showed a community profile more similar to health. These results indicated that the whole community of severe naturally occurring gingivitis, G-HBI, may have shifted more towards early periodontitis than G-LBI. The dysbiosis state of the subgingival community may already exist in severe gingivitis before the periodontal lesion is established.

Furthermore, the community structure of G-LBI and G-HBI was significantly different even though they had a similar PI, indicating that in gingivitis, the severity of disease (BI) is associated with the subgingival microbial community. Previous studies on experimental gingivitis also found community shifts correlating with clinical signs of gingivitis[10,11,21]. In our data, the only detected differential abundant taxon between these two subgroups was *P. gingivalis*. Emerging evidence has supported that *P. gingivalis*, acting as a 'keystone

pathogen', can modulate the host response by impairing immune surveillance and altering the balance from symbiosis to dysbiosis[38]. *P. gingivalis* can also increase the virulence of the whole microbial community through interaction with commensals[39]. On the other hand, the local environmental factors of gingival tissue, such as temperature and hemin concentrations, also modulate the virulence structures of *P. gingivalis*, thus influencing its capability to modulate immune response[40,41]. Collectively, in our data, the difference in severity of gingivitis accompanied by the distinct community signature in gingivitis patients may reflect the interaction between the disease-provoking community and host immune response. Importantly, the distinct abundance of *P. gingivalis* in G-LBI and G-HBI may suggest that even in gingivitis, *P. gingivalis* may have already played its role as a keystone pathogen and may increase susceptibility to periodontitis in patients with severe gingivitis.

In our study, healthy individuals harboured a higher proportion of *P. nigrescens* than the other groups. An increase in *P. nigrescens* was also found in the periodontally healthy group in an elderly population[19] and naturally occurring gingivitis after intervention[42]. However, *P. nigrescens* was a member of the "orange complex" correlating with periodontitis[3]. A recent study found that *P. nigrescens* from diseased samples had different virulence factors from healthy samples[43], which may partly explain this discrepancy.

In the present study, we chose the interproximal sites of teeth 16, 26, 36, 46, 11 and 31 as sites to be sampled. Previous epidemiological studies in young adults demonstrated that first molars and incisors were more affected by periodontitis than other teeth[44,45]. The community periodontal index of treatment needs (CPITN) recommended teeth 16, 26, 36, 46, 11 and 31 for periodontitis screening for individuals aged under 20 years[46]. In the present study, due to funding limitations and the relatively small amount of plaque from the subgingival area, the samples from each participant were pooled. Potential bias may have arisen from a dilution effect from healthy sites in the pooled samples, and this could be considered a limitation of the present study; however, even though potential dilution was considered, the microbial features of early periodontitis still differed from the gingivitis and health groups. Furthermore, in order to control the potential influence of age and geographic factors on the oral microbiome[47,48], all the participants were aged between 18 and 21 years and were from the same geographic area; however, such a convenient sample might give rise to difficulties in extrapolating the conclusions to

other populations. In addition, the sample size of the present study was relatively small, especially in the periodontitis and health groups. Further larger-scale studies examining different age groups and geographical areas are necessary.

Conclusion

Within the limitations of the present study, gingivitis and early-stage periodontitis were associated with an increased degree of dysbiosis of subgingival microbiota in a Chinese young adult population. Future longitudinal studies combining bacterial function and host immune response in such a population would further elucidate the pathogenesis of initial periodontitis.

Conflicts of interest

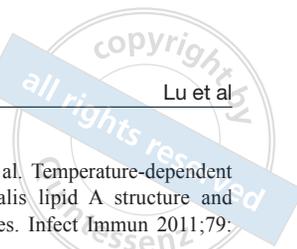The authors declare no conflicts of interest related to this study.

Author contribution

Drs Chang LU and Yi CHU conducted the study and prepared the manuscript; Drs Jian Ru LIU and Wen Yi LIU revised the manuscript; Prof Xiang Ying OUYANG designed and supervised the study and revised the manuscript.

References

1. Löe H, Theilade E, Jensen SB. Experimental gingivitis in man. J Periodontol 1965;36:177–187.
2. Lang NP, Schätzle MA, Löe H. Gingivitis as a risk factor in periodontal disease. J Clin Periodontol 2009;36(suppl 10):3–8.
3. Socransky SS, Haffajee AD, Cugini MA, Smith C, Kent RL Jr. Microbial complexes in subgingival plaque. J Clin Periodontol 1998;25:134–144.
4. Moore WE, Moore LV. The bacteria of periodontal diseases. Periodontol 2000 1994;5:66–77.
5. Holt SC, Ebersole JL. Porphyromonas gingivalis, Treponema denticola, and Tannerella forsythia: The "red complex", a prototype polybacterial pathogenic consortium in periodontitis. Periodontol 2000 2005;38:72–122.
6. Dewhirst FE, Chen T, Izard J, et al. The human oral microbiome. J Bacteriol 2010;192:5002–5017.
7. Huang S, Yang F, Zeng X, et al. Preliminary characterization of the oral microbiota of Chinese adults with and without gingivitis. BMC Oral Health 2011;11:33.
8. Griffen AL, Beall CJ, Campbell JH, et al. Distinct and complex bacterial profiles in human periodontitis and health revealed by 16S pyrosequencing. ISME J 2012;6:1176–1185.
9. Abusleme L, Dupuy AK, Dutzan N, et al. The subgingival microbiome in health and periodontitis and its relationship with community biomass and inflammation. ISME J 2013;7:1016–1025.
10. Kistler JO, Booth V, Bradshaw DJ, Wade WG. Bacterial community development in experimental gingivitis. PLoS One 2013;8:e71227.
11. Huang S, Li R, Zeng X, et al. Predictive modeling of gingivitis severity and susceptibility via oral microbiota. ISME J 2014;8:1768–1780.
12. Kirst ME, Li EC, Alfant B, et al. Dysbiosis and alterations in predicted functions of the subgingival microbiome in chronic periodontitis. Appl Environ Microbiol 2015;81:783–793.
13. Park OJ, Yi H, Jeon JH, et al. Pyrosequencing analysis of subgingival microbiota in distinct periodontal conditions. J Dent Res 2015;94:921–927.
14. Deng K, Ouyang XY, Chu Y, Zhang Q. Subgingival microbiome of gingivitis in Chinese undergraduates. Chin J Dent Res 2017;20: 145–152.
15. Tsai CY, Tang CY, Tan TS, Chen KH, Liao KH, Liou ML. Subgingival microbiota in individuals with severe chronic periodontitis. J Microbiol Immunol Infect 2018;51:226–234.
16. Pérez-Chaparro PJ, McCulloch JA, Mamizuka EM, et al. Do different probing depths exhibit striking differences in microbial profiles? J Clin Periodontol 2018;45:26–37.
17. Chen WP, Chang SH, Tang CY, Liou ML, Tsai SJ, Lin YL. Composition analysis and feature selection of the oral microbiota associated with periodontal disease. Biomed Res Int 2018;2018:3130607.
18. Genco RJ, LaMonte MJ, McSkimming DI, et al. The subgingival microbiome relationship to periodontal disease in older women. J Dent Res 2019;98:975–984.
19. Papapanou PN, Park H, Cheng B, et al. Subgingival microbiome and clinical periodontal status in an elderly cohort: The WHICAP ancillary study of oral health. J Periodontol 2020;91(suppl 1):S56–S67.
20. Hajishengallis G, Lamont RJ. Beyond the red complex and into more complexity: The polymicrobial synergy and dysbiosis (PSD) model of periodontal disease etiology. Mol Oral Microbiol 2012;27: 409–419.
21. Schincaglia GP, Hong BY, Rosania A, et al. Clinical, immune, and microbiome traits of gingivitis and peri-implant mucositis. J Dent Res 2017;96:47–55.
22. Tanner A, Maiden MF, Macuch PJ, Murray LL, Kent RL Jr. Microbiota of health, gingivitis, and initial periodontitis. J Clin Periodontol 1998;25:85–98.
23. Diaz PI, Hoare A, Hong BY. Subgingival microbiome shifts and community dynamics in periodontal diseases. J Calif Dent Assoc 2016;44:421–435.
24. Tonetti MS, Greenwell H, Kornman KS. Staging and grading of periodontitis: Framework and proposal of a new classification and case definition. J Periodontol 2018;89(suppl 1):S159–S172.
25. Chapple ILC, Mealey BL, Van Dyke TE, et al. Periodontal health and gingival diseases and conditions on an intact and a reduced periodontium: Consensus report of workgroup 1 of the 2017 World Workshop on the Classification of Periodontal and Peri-Implant Diseases and Conditions. J Periodontol 2018;89(suppl 1):S74–S84.
26. Mazza JE, Newman MG, Sims TN. Clinical and antimicrobial effect of stannous fluoride on periodontitis. J Clin Periodontol 1981;8: 203–212.
27. Silness J, Löe H. Periodontal disease in pregnancy II. Correlation between oral hygiene and periodontal condition. Acta Odontol Scand 1964;22:121–135.
28. Caporaso JG, Lauber CL, Walters WA, et al. Ultra-high-throughput microbial community analysis on the Illumina HiSeq and MiSeq platforms. ISME J 2012;6:1621–1624.
29. Edgar RC. UPARSE: Highly accurate OTU sequences from microbial amplicon reads. Nat Methods 2013;10:996–998.

30. Quast C, Pruesse E, Yilmaz P, et al. The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. Nucleic Acids Res 2013;41(Database issue):D590–D596.
31. Mandal S, Van Treuren W, White RA, Eggesbø M, Knight R, Peddada SD. Analysis of composition of microbiomes: A novel method for studying microbial composition. Microb Ecol Health Dis 2015;26:27663.
32. Weiss S, Xu ZZ, Peddada S, et al. Normalization and microbial differential abundance strategies depend upon data characteristics. Microbiome 2017;5:27–44.
33. Tanner AC, Paster BJ, Lu SC, et al. Subgingival and tongue microbiota during early periodontitis. J Dent Res 2006;85:318–323.
34. Liu P, Qiu Q, Lu Y. Syntrophomonadaceae-affiliated species as active butyrate-utilizing syntrophs in paddy field soil. Appl Environ Microbiol 2011;77:3884–3887.
35. Wu YF, Xing P, Liu S, Wu QL. Enhanced microbial interactions and deterministic successions during anoxic decomposition of microcystis biomass in lake sediment. Front Microbiol 2019;10:2474.
36. Gomes BP, Berber VB, Kokaras AS, Chen T, Paster BJ. Microbiomes of endodontic-periodontal lesions before and after chemomechanical preparation. J Endod 2015;41:1975–1984.
37. Huang CB, Alimova Y, Myers TM, Ebersole JL. Short- and medium-chain fatty acids exhibit antimicrobial activity for oral microorganisms. Arch Oral Biol 2011;56:650–654.
38. Hajishengallis G, Darveau RP, Curtis MA. The keystone-pathogen hypothesis. Nat Rev Microbiol 2012;10:717–725.
39. Hajishengallis G, Liang S, Payne MA, et al. Low-abundance biofilm species orchestrates inflammatory periodontal disease through the commensal microbiota and complement. Cell Host Microbe 2011;10:497–506.
40. Curtis MA, Percival RS, Devine D, et al. Temperature-dependent modulation of Porphyromonas gingivalis lipid A structure and interaction with the innate host defenses. Infect Immun 2011;79:1187–1193.
41. Al-Qutub MN, Braham PH, Karimi-Naser LM, Liu X, Genco CA, Darveau RP. Hemin-dependent modulation of the lipid A structure of Porphyromonas gingivalis lipopolysaccharide. Infect Immun 2006;74:4474–4485.
42. Stone SJ, Kumar PS, Offenbacher S, Heasman PA, McCracken GI. Exploring a temporal relationship between biofilm microbiota and inflammatory mediators during resolution of naturally occurring gingivitis. J Periodontol 2019;90:627–636.
43. Zhang Y, Zhen M, Zhan Y, Song Y, Zhang Q, Wang J. Population-genomic insights into variation in Prevotella intermedia and Prevotella nigrescens isolates and its association with periodontal disease. Front Cell Infect Microbiol 2017;7:409.
44. Clerehugh V, Lennon MA, Worthington HV. 5-year results of a longitudinal study of early periodontitis in 14- to 19-year-old adolescents. J Clin Periodontol 1990;17:702–708.
45. Susin C, Haas AN, Valle PM, Oppermann RV, Albandar JM. Prevalence and risk indicators for chronic periodontitis in adolescents and young adults in south Brazil. J Clin Periodontol 2011;38:326–333.
46. Cutress TW, Ainamo J, Sardo-Infirri J. The community periodontal index of treatment needs (CPITN) procedure for population groups and individuals. Int Dent J 1987;37:222–233.
47. Haffajee AD, Bogren A, Hasturk H, Feres M, Lopez NJ, Socransky SS. Subgingival microbiota of chronic periodontitis subjects from different geographic locations. J Clin Periodontol 2004;31:996–1002.
48. Huang S, Haiminen N, Carrieri AP, et al. Human skin, oral, and gut microbiomes predict chronological age. mSystems 2020;5:e00630-19.