Cellular Physiology and Biochemistry

Cell Physiol Biochem 2018;47:774-783
DOI: 10.1159/000490029
Published online: May 28, 2018
Accepted: February 12, 2018

Original Paper

Comparative Analyses of Subgingival Microbiome in Chronic Periodontitis Patients with and Without IgA Nephropathy by High Throughput 16S rRNA Sequencing

Yali Cao[a,b] Min Qiao[c] Zhigang Tian[d] Yan Yu[e] Baohua Xu[c] Wansheng Lao[a] Xuguo Ma[a] Wenge Li[b]

[a]Department of Nephrology, the Third People's Hospital of Longgang, Shenzhen, [b]Department of Nephrology, China-Japan Friendship Hospital, Beijing, [c]Department of Stomatology, China-Japan Friendship Hospital, Beijing, [d]Beijing Guangdalongtai Polytron Technologies Inc, Beijing, [e]Allwegene Tech., Beijing, China

Key Words
Chronic periodontitis • IgA nephropathy • Subgingival microbiome • Barcoded MiSeq sequencing • 16S rRNA gene

Abstract
Background/Aims: Periodontitis is a prevalent chronic inflammatory disease caused by enhanced inflammation induced by dysbiotic microbes forming on subgingival tooth sites, which may disturb the balance of the microbial composition in the biofilm and finally result in the progressive destruction of the periodontal ligament and alveolar bone with periodontal pocket formation and/or gingival recession. ***Methods:*** To elucidate the correlation between subgingival microbiome and IgAN incidence in CP (chronic periodontitis at severe levels) patients, subgingival plaque samples were collected from CP patients without IgAN (Control) and CP patients with IgAN (Disease). 16S rRNA sequencing and comparative analyses of plaque bacterial microbiome between Control and Disease were performed**.** ***Results:*** Subgingival microbial diversity in Disease was a little higher than that in Control. Besides, significant differences were found in subgingival microbiome between Disease and Control. Compared with that in Control, at phylum level, the abundances of Proteobacteria and Actinobacteria were significantly higher while the abundances of Bacteroidetes, Fusobacteria, Spirochaetae, Synergistetes, and Saccharibacteria were significantly lower in Disease; at class level, the abundances of Betaproteobacteria, Bacilli, Actinobacteria, Flavobacteriia, and Gammaproteobacteria were significantly higher while the abundances of Bacteroidia, Fusobacteriia, Negativicutes, Clostridia, and Spirochaetes were significantly lower in Disease;

Yali Cao
Department of Nephrology, the Third People's Hospital of Longgang
No. 278 Songbai Road, Longgang District, Shenzhen 518115 (China)
Tel. +86-755-28863118, Fax +86-755-28863118; E-Mail caoyali151108@163.com

at genus level, the abundances of *Bergeyella*, *Capnocytophaga*, *Actinomyces*, *Corynebacterium*, *Comamonas*, *Lautropia*, and *Streptococcus* were significantly higher while the abundances of *Treponema* and *Prevotella* were significantly lower in Disease. ***Conclusions:*** Our data indicated a correlation between the changes in subgingival microbial structure and IgAN incidence in CP patients, which might be used to predict IgAN incidence in CP patients.

Introduction

In China, 80-90 percent of adults have periodontal problems, such as tartar, plaque, bleeding gums, and periodontitis, and about 15-20 percent of patients have chronic periodontitis at severe levels (CP) [1, 2]. The periodontitis incidence in China is higher than that in other countries, especially CP [2]. Periodontitis is a prevalent chronic inflammatory disease caused by enhanced inflammation induced by dysbiotic microbes forming on subgingival tooth sites, which may disturb the balance of the microbial composition in the biofilm and finally result in the progressive destruction of the periodontal ligament and alveolar bone with periodontal pocket formation and/or gingival recession [3]. The mechanism underlying destruction of periodontal tissues includes tissue damages caused by plaque bacterial products and bacterial induction of the host immune responses [3]. Periodontitis is considered as a leading cause of tooth loss in adults and historically viewed separately from those of the rest of the body [4, 5]. However, recent studies indicate that periodontal infection is a constant potential source of infection and is associated with numerous systemic diseases, including atherosclerosis, diabetes, cancer, rheumatoid arthritis, aspiration pneumonia, and adverse pregnancy outcomes [5-19]_ENREF_2. The mechanisms or pathways linking oral infections to secondary systemic effects are infections from oral cavity via transient bacteremia, injury caused by circulating oral microbial toxins, and inflammation caused by immunological injury induced by oral microorganisms. Therefore, promotion of oral health has been suggested as a way to promote systemic health [20].

IgA nephropathy (IgAN), also known as Berger's disease, is a chronic glomerular disease that occurs when IgA deposits in the glomerular mesangium [21, 22]. IgAN usually progresses slowly over many years, and patients with IgAN usually present with proteinuria or microscopic hematuria, alone or in combination [23]. This results in local inflammation that may hamper kidneys' function. About 25 % of adults with IgAN develop total kidney failure. A recent study indicates that the microbiome in IgAN is changed, such as a decrease in *Clostridium*, *Enterococcus*, *Lactobacillus*, *Leuconostoc,* and *Bifidobacterium,* and an increase in *Streptococcus sp.* and *Firmicutes* [24]. Till now, the relationship between periodontal infection and IgAN is not well studied. Our previous study has reported that the prevalence of CP and aggressive periodontitis in IgAN patients is higher than that in non-IgAN patients ($P<0.05$), indicating that periodontitis is correlated with the onset and development of IgAN [25]. To further investigate the correlation between plaque bacterial microbiomes and IgAN incidence in CP patients, subgingival plaque samples from CP patients with or without IgAN were collected and comparative analyses of plaque bacterial microbiomes were further performed by using high throughput 16S rRNA sequencing.

Materials and Methods

Ethics Statement

Written informed consent was obtained from all the patients in this study. The study design, protocol, and informed consent were approved by Ethics Committee of China-Japan Friendship Hospital (2013-KY-3). The methods were carried out in accordance with relevant guidelines.

Collection of subgingival plaque samples

A total of 20 patient samples that included 9 CP patients without IgAN (Control) and 11 CP patients with IgAN (Disease) were selected and examined at China-Japan Friendship Hospital. Briefly, patients at the dental clinic and nephrology clinic without any treatment were selected for further sample collection. Subgingival plaque samples were collected from the first molars by means of a sterile excavating-spoon hand-instrument and then placed immediately into an Eppendorf tube containing 1 ml of sodium thiosulfate solution. Meanwhile, the clinical diagnoses of periodontal disease and renal disease were performed. Samples collected from patients with CP were considered as Control and samples collected from patients with CP and IgAN were considered as Disease.

Table 1. Barcode sequence information.

Sample ID	F-barcodes	R-barcodes
C1	GAGCTGAC	TTAACACA
C2	TGATGGCT	CGGCACCT
C3	TGAATCAT	TACTTCGC
C4	TGACAGAC	AGGCAGAC
C5	GTGGTCGT	TCTACTCC
C6	GCGTGGAG	GCTGCGCA
C7	ACTTCCGC	CACTAGCA
C8	ACATGTAC	GAGCTCGG
C9	CCGGCTAA	CTAATCCG
D1	CGATCCTG	CCTCAGTC
D2	TAATTACC	GCTTACGA
D3	ATAACACC	CGTGACGG
D4	CGTAGGAC	TACTTCGC
D5	CTCTCGAC	CGCAGTCC
D6	TCGGTCAC	TGAACCTC
D7	CTACGCAC	TTGTACTC
D8	AGGTTAAC	CAATGCTC
D9	GTTGCAAC	CACGGCGA
D10	CTCAATTA	CGCCGCTG
D11	CAAGTCTA	GCATCCTT

Generation of amplicon libraries and Miseq sequencing

Genomic DNA was extracted from subgingival samples using the PowerSoil® DNA isolation kit (MO BIO Laboratories, Inc. Cat. 12888-100) according to the manufacturer's instructions. The amount of total DNA was determined using Nanodrop ND-2000 (ThermoScientific, Wilmington, DE, USA). Integrity and size of DNA were checked by 1% (w/v) agarose gel electrophoresis. The V3-4 hypervariable regions of bacterial 16S rRNA gene were amplified with the primers 357F (5'-CCTACGGGAGGCAGCAG-3') and 806R (5'-GGACTACHVGGGTWTC TAAT-3'). For each sample, 10-digit barcode sequence (Table 1) was added to the 5' end of the forward and reverse primers (provided by Auwigene Company, Beijing) for differing each sample. PCR was carried out on a Mastercycler Gradient (Eppendorf, Germany) using 50 μl reaction volumes, containing 5 μl 10×Ex Taq Buffer (Mg^{2+} plus), 4 μl 12.5 mM dNTP Mix (each), 1.25 U Ex Taq DNA polymerase, 2 μl template DNA, 200 nM barcoded primers 357F and 806R each, and 36.75 μl ddH_2O. Cycling parameters were 94 °C for 2 min, followed by 30 cycles of 94 °C for 30 s, 57 °C for 30 s, and 72 °C for 30 s with a final extension at 72 °C for 10 min. Three PCR products per sample were pooled to mitigate reaction-level PCR biases. The PCR products were purified using a QIAquick Gel Extraction Kit (QIAGEN, Germany), quantified using Real-Time PCR, and sequenced at Auwigene Company, Beijing.

Data processing and statistical analyses

Raw sequencing data were processed by Beijing Auwigene Tech, Ltd. (Beijing, China) using the pipeline tools QIIME and MOTHUR. The overlapping paired-end reads were merged using FLASH (v 1.2.10). The sequences were removed from consideration if they were shorter than 200 bp, had a low-quality score (≤ 20), contained ambiguous bases or did not exactly match to primer sequences and barcode tags using Trimmomatic. Filtered reads were sorted into different samples according to their barcodes with MOTHUR. The retained high-quality sequences were further analyzed using MOTHUR and Usearch (version 8.0.1623). Specifically, MOTHUR was used for barcode and primer sequence removal, 'trim.seqs (maxhomop = 10, minlength = 200)' was used for quality filtering, and Usearch (version 8.0.1623) was used for de novo removal of chimeric reads. All the clean tags of all samples were clustered into OTUs using QIIME (v1.9.1) at 97% sequence similarity. These OTUs were used as a basis for calculating alpha-diversity and beta-diversity metrics using QIIME (v1.9.1). The sufficiency of the sampling effort was evaluated by drawing rarefaction curves, the bacterial community diversity within each individual sample was estimated using the Shannon-Wiener index, the species richness was estimated with the CHAO1 index, and the percentage of coverage was calculated by Good's coverage estimator. The Ribosomal Database Project (RDP) Classifier tool was used to classify all sequences into different taxonomic groups.

The beta-diversity was performed with QIIME (v1.9.1) to assess the differences of microbial communities between Control and Disease based on their composition. A principal coordinate analysis (PCoA) of weighted UniFrac was performed to compare the overall structure of subgingival microbiome of all samples, based on the relative abundance of OTUs (at a 97% similarity level). The abundance of bacterial phyla and genus for each group was expressed as the percentage of total sequences and the bacterial

community structures of Control and Disease were further compared at phylum and genus level using Mann–Whitney U test. P-values were corrected using a false discovery rate (FDR) correction to account for correction of multiple testing [26]. The statistical differences between Control and Disease were analyzed using ANOVA according to the methods provided by Mothur.

Results

Demographic and clinical characteristics of studied subjects

Subgingival plaque samples were collected from 9 CP patients without IgAN (Control) and 11 CP patients with IgAN (Disease). For Control and Disease, the average age were 37.3 and 36.1 years old, respectively; the probing depths (PD) were 4.8 mm and 5.2 mm, respectively; the clinical attachment levels (CAL) were 5.1 mm and 4.9 mm, respectively; and the percentage of surfaces of the plaque were 85.2 and 82.9, respectively (Table 2). No statistical differences were found in these parameters between Control and Disease.

Characteristics of MiSeq sequencing results

In total, 408, 758 raw reads were obtained from all 20 subgingival plaque samples. After filtering, 350773 filtered clean tags (17538.65 tags per sample) and 8795 OTUs (439.75 OTUs per sample) were obtained from all the samples (Table 3). The Shannon-Wiener curve of all samples already reached a plateau at this sequencing depth (Fig. 1) and the coverage was higher than 95%, suggesting that the sequencing was deep enough. Though there were no statistically significant differences in the community richness estimator (Chao) and

Table 2. Demographic and clinical characteristics of studied subjects. P>0.05. CAL, clinical attachment levels; PD, probing depths. CAL and PD were measured in mm and represent the mean for all sites in the oral cavity of studied subjects.

Characteristic	Control(n=9)	Disease(n=11)
M/F	5/4	6/5
Age (years±s.d.)	37.3±2.9	36.1±3.8
PD (mean±s.d.)	4.8±0.9	5.2±0.6
CAL (mean±s.d.)	5.1±0.5	4.9±0.3
% surfaces with plaque (mean±s.d.)	85.2±10.1	82.9±9.8

Table 3. Number of raw tags, clean tags, final tags, and OTUs in Control and Disease by 16S rRNA sequencing.

Sample ID	Raw Tags	Clean Tags	Final Tags	OTU
C1	18241	16752	16284	250
C2	20797	18042	17402	396
C3	20366	18083	17429	485
C4	18740	17674	17255	311
C5	20755	17969	17289	466
C6	21059	18824	18017	503
C7	21577	18624	17927	417
C8	24932	19222	18133	460
C10	21084	19668	19135	342
D1	18532	17981	17439	441
D3	20724	17965	16994	540
D6	17598	14522	13651	497
D7	21342	17413	16326	632
D8	20644	16141	15252	495
D9	19389	16202	15249	511
D10	20681	17012	16224	366
D11	18955	13536	13036	400
D12	20815	17455	16762	287
D13	20888	17789	16771	636
D14	21639	19899	19136	360
Average	20437.9	17538.65	16785.55	439.75

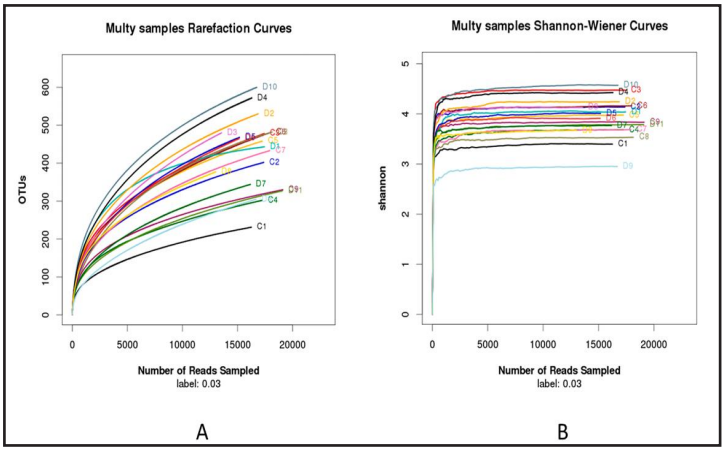


Fig. 1. Rarefaction curves and Shannon-Wiener curves of each subgingival sample collected from 9 periodontitis patients without IgAN and 11 periodontitis patients with IgAN.

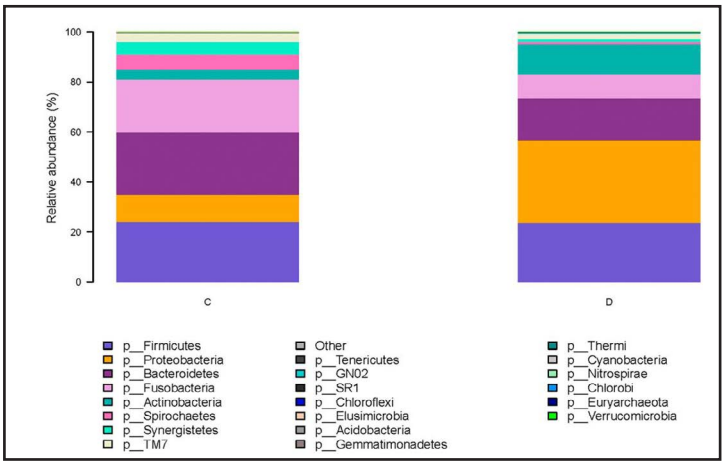


Fig. 2. Relative abundance of bacteria in subgingival microbiome of Control and Disease at phylum level. Control, periodontitis patients without IgA nephropathy; Disease, periodontitis patients with IgA nephropathy.

the diversity estimator (Shannon index) between Control and Disease (p=0.34 and 0.59, respectively), the diversity indices in Disease were a little higher than that in Control (Table 4).

Taxonomy at phylum, class and genus levels in Control and Disease

Overall bacterial compositions for each group at phylum level were shown in Fig. 2 and Table 5. In general, a total of 23 phyla in all samples were obtained. The dominant phyla of all groups were Firmicutes, Proteobacteria, Bacteroidetes, and Fusobacteria, accounting for 81.79% of the total sequences. The most dominant phyla were Firmicutes in Control and Proteobacteria in Disease. In addition, the microbial composition, belonging to Proteobacteria and Bacteroidetes, varied greatly between Control and Disease ($p<0.05$). Though no significant differences were found, the abundance of Actinobacteria was higher while the abundances of Bacteroidetes, Fusobacteria, Spirochaetae, and Synergistetes were lower in the subgingival microbiome of Disease than that in Control (Fig. 1). Overall microbial compositions for each group at class level were shown in Fig. 3 and Table 6. There were 37 classes in all samples. The dominant classes of both groups were Bacteroidia, Fusobacteriia, Negativicutes, Clostridia,

Table 4. Summary of MiSeq sequencing data. The number of reads, OTUs, richness estimator Chao, and diversity estimator Shannon were calculated at the 97% similarity level. Control, periodontitis patients without IgA nephropathy; Disease, periodontitis patients with IgA nephropathy.

Group	M/F	Age (year)	OTUs	Chao	Coverage	Shannon
Control	5/4	39±4	482±79	930±192	0.98±0.03	5.49±0.63
Disease	6/5	38±3	573±138	1030±248	0.97±0.00	5.65±0.70

Table 5. Relative abundance at the phylum level and statistical significance between Control and Disease. Control, periodontitis patients without IgA nephropathy; Disease, periodontitis patients with IgA nephropathy. Significance: NS > 0.05, *< 0.05, **< 0.01, ***< 0.001.

phyla	Control(n=9)	Disease(n=11)	p value
Proteobacteria	11.25±0.05.67	33.54±11.94	0.0015
Bacteroidetes	25.32±0.06.87	17.23±7.11	0.0030
Gracilibacteria	0.01±0.00.03	0.14±0.12	0.0083
Spirochaetae	6.13±0.04.76	1.01±1.21	0.0201
Fusobacteria	20.40±0.08.09	8.96±5.08	0.0252
Actinobacteria	4.00±0.03.53	11.62±14.64	0.0387

Table 6. Relative abundance at class level and statistical significance between Control and Disease. Control, periodontitis patients without IgA nephropathy; Disease, periodontitis patients with IgA nephropathy. Significance: NS > 0.05, *< 0.05, **< 0.01, ***< 0.001.

Class	Relative abundance (%)		p value
	Control	Disease	Control vs Disease
Bacteroidia	22.26±0.08.15	9.46±0.07.12	0.0002
Flavobacteriia	2.64±0.02.58	7.67±0.03.55	0.0073
Betaproteobacteria	6.73±0.05.60	23.08±0.11.40	0.0073
Bacilli	3.49±0.03.86	12.37±0.08.63	0.0159
Spirochaetes	6.13±0.04.76	1.01±0.01.21	0.0201
Fusobacteriia	20.40±0.08.09	8.96±0.05.08	0.0252
Gammaproteobacteria	2.38±0.01.62	7.18±0.03.68	0.0252
Clostridia	7.00±0.03.29	3.20±0.01.86	0.0314
Actinobacteria	3.85±0.03.52	11.44±0.14.73	0.0387
Sphingobacteriia	0.42±0.00.69	0.09±0.00.12	0.0401

Betaproteobacteria, Spirochaetes, Synergistia, Actinobacteria, Bacilli, and Flavobacteriia, representing 93% of total sequences. The most dominant class were Bacteroidia in Control and Betaproteobacteria in Disease. However, there were no significant differences between these two groups. At order level, a total of 70 orders were detected. Bacteroidales, Fusobacteriales, Neisseriales, Selenomonadales, and Lactobacillales represented 58.61 % of the total sequences. The abundance of Bacteroidales in Control (22.26%) was much higher than that in Disease (9.46%) while the abundance of Burkholderiales in Disease (8.23%) was much higher than that in Control (0.86%). At genus level, the abundances of *Treponema_2* and *Prevotella* were lower while the abundances of *Bergeyella, Lautropia, Actinomyces, Comamonas, Corynebacterium, Capnocytophaga*, and *Streptococcus* were higher in Disease than that in Control (Fig. 4).

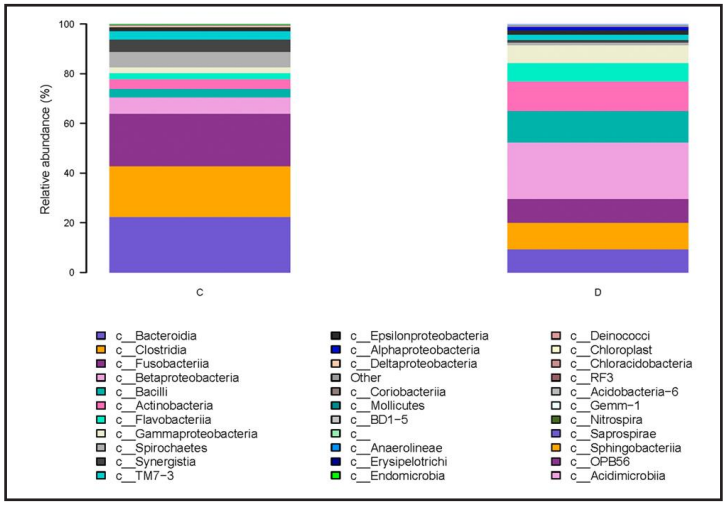


Fig. 3. Relative abundance of bacteria in subgingival microbiome of Control and Disease at class level. Control, periodontitis patients without IgA nephropathy; Disease, periodontitis patients with IgA nephropathy.

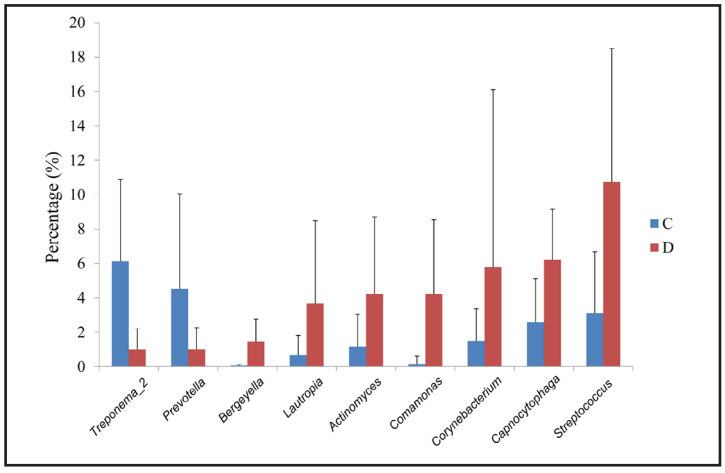
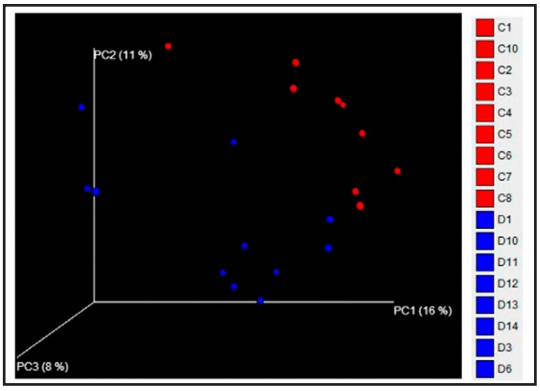


Fig. 4. Relative abundance of bacteria in subgingival microbiome of Control and Disease at genus level. Control, periodontitis patients without IgA nephropathy; Disease, periodontitis patients with IgA nephropathy.

Fig. 5. Weighted UniFrac measures of beta-diversity visualized using principal coordinate analysis (PCoA). Control, periodontitis patients without IgA nephropathy; Disease, periodontitis patients with IgA nephropathy.



Beta-diversity of subgingival microbiome between Control and Disease with multivariate statistics analysis

PCoA based on the compositions of OTUs in each sample was performed to compare the overall structure of subgingival microbiome between these two groups. There was an obvious separation of Control and Disease, PC1and PC2 accounted for 22.14% and 13.74% of the total variations, respectively (Fig. 5). Statistical analyses indicated that microbial composition of Disease was significantly different from that of Control ($p<0.001$).

Discussion

16S rRNA Miseq platform was used for assessing the relationship between the bacterial community and IgAN. The results showed that bacterial diversity in Disease was higher than that in Control. Some bacterial groups in Disease were also significantly different from that in Control. The structure of bacteria between the two groups varied significantly.

Periodontal disease is a prevalent chronic inflammatory disease of the oral cavity which is the major cause of tooth loss in adults [27]. Almost all forms of periodontal disease occur due to mixed microbial infections and many bacterial species are recognized as putative periodontal pathogens, such as *Porphyromonas gingivalis, Aggregatibacter actinomycetemcomitans, Bacteroides forsythus, Prevotella intermedia, Peptostreptococcus micros, Fusobacterium nucleatum, Filifactor alocis*, *Desulfobulbus sp. oral taxon 041* HOT 041, and Synergistetes [28-31]. Recent studies have indicated that periodontal disease and systemic health are closely linked and treating periodontal disease may help with the prevention of several other chronic inflammatory conditions, including IgAN [6, 16-19, 25]. IgAN is the most common primary glomerulonephritis wherein immune complexes consisting of IgA1 with galactose-deficient hinge region and anti-glycan antibodies deposit in glomeruli and induce renal injury [32]. Till now, knowledge about the impact of subgingival microbiome in CP patients on IgAN incidence is still limited. In this study, we used 16S rRNA sequencing to identify and compare bacteria present within Control and Disease and further investigated the correlation between subgingival microbiome and IgAN incidence in CP patients. Study subjects in Control and Disease were carefully selected, such that no statistically significant differences were present in average ages, probing depths, and clinical attachment levels. Moreover, statistically significant differences were found in subgingival microbial communities but not found in diversity.

The subgingival microbiome of Disease was compositionally distinct from that of Control. Disease had a higher relative abundance of phyla Proteobacteria and Actinobacteria, and a lower relative abundance of phyla Bacteroidetes, Fusobacteria, Spirochaetae, Synergistetes, and Saccharibacteria. Compared to Control, the ratio between Firmicutes/Proteobacteria markedly decreased in Disease, which is consistent with the previous study that has demonstrated decreasing ratio between Firmicutes/Proteobacteria in the salivary microbiome of IgAN patients [33]. The changes of phyla Proteobacteria, Actinobacteria, Bacteroidetes, Fusobacteria, and Spirochaetae in Disease were mainly due to significant changes in class Betaproteobacteria and Gammaproteobacteria, Actinobacteria, Bacteroidia, Fusobacteriia, and Spirochaetes, respectively. Further comparison analysis at genus level indicated that compared to Control, genera *Treponema* and *prevotella* were less but *Bergeyella*, *Lautropia*, *Actinomyces*, *Comamonas*, *Corynebacterium*, *Capnocytophaga*, and *Streptococcus* were more abundant in Disease. The genus *Treponema* belongs to the phylum Spirochaetes. As reported, *treponemes* are involved in the etiology of chronic periodontitis and other forms of periodontal disease [34]. *Treponema denticola* resides in the human oral cavity and is highly correlated with the incidence and severity of human periodontal diseases [35, 36]. The genus *Prevotella* belongs to the phylum Bacteroidetes. A variety of *Prevotella spp., including P. melaninogenica*, *P. intermedia*, and *P. loescheii*, reside in the human oral cavity [37, 38]. *P. intermedia* might be a periodontal pathogen, whereas *P. nigrescens* is a marker of relative periodontal health [37]. Besides, it is found that, in the salivary microbiome,

the relative abundances of *Prevotella spp.* (*P. nigrescens*, *P. intermedia*, *P. pallens*, and *P. salivae*) were higher in health control compared to IgAN patients and the only exception was *P. aurantiaca* [33]. Our data also indicated that higher abundance of *Prevotella spp.* was found in Control compared to Disease. The genus *Bergeyella* is a hard-to-cultivate taxon belonging to the phylum Bacteroidetes. The information regarding the role of *Bergeyella* in periodontal disease and IgAN is very limited [39]. The genus *Lautropia* belongs to the phylum Proteobacteria. Only one species, *L. mirabilis*, has been identified and was found to be associated with success in periodontal therapy [40]. The genus *Actinomyces* belongs to the phylum Actinobacteria (except *A. meyeri*, an obligate anaerobe). A previous study found that *Actinomyces odontolyticus/meyeri and Actinomyces israelii* were associated with chronic periodontitis (p=0.003) [41]. The genus *Comamonas* belongs to the phylum Proteobacteria. No information is available regarding the role of *Comamonas* in periodontal disease and IgAN. The genus *Corynebacterium* belongs to the phylum Actinobacteria. A previous study found that *Corynebacterium diphtheria* was in higher prevalence and level in the subgingival biofilm samples collected from patient with periodontitis, which is consistent with our data [42]. Piccolo, M. *et al.* found that the abundance of *Corynebacterium* sp. was lower in the salivary samples of IgAN patients compared to that in healthy control, showing an opposite conclusion [33]. The genus *Capnocytophaga* belongs to the phylum Bacteroidetes. *Capnocytophaga spp.* are often isolated from periodontal pockets, apical, and periodontal abscesses. It is found that *Capnocytophaga spp.* are more prevalent in gingivitis compared to healthy periodontium and periodontitis. *Capnocytophaga spp.* have the potential to cause periodontal disease [43-46]. The genus *Streptococcus* belongs to the phylum Firmicutes. In 2011, Huang *et al.* studied the relationship between oral microbiome and gingivitis status and found that *Streptococcus* was associated with gingivitis [47]. It is reported that *Streptococcus* has a correlation with IgAN [48]. A link between tonsillar infection caused by *Streptococcus* sp. and IgAN was hypothesized. Besides, streptococcal proteins were recognized by sera of IgAN patients [48, 49]. In the present study, *Streptococcus* was also upregulated in Disease, implying that *Streptococcus spp.* in CP patients has a correlation with IgAN.

In summary, our data indicated statistically significant changes in the subgingival microbiome in Disease compared to that in Control, implying a correlation between the changes in subgingival microbial structure and IgAN incidence in CP patients. However, till now, the knowledge regarding the roles of the changed subgingival microbial structure of CP patients in IgAN incidence is still limited and further investigation is needed.

Acknowledgements

This study was supported by Beijing Nova Program (No. xx2014B074).

Disclosure Statement

The authors declare to have no competing interests.

References

1 Cao C, Meng H: [current status of the research of periodontal diseases in china]. Zhonghua kou qiang yi xue za zhi = Chin J Stomatol 1997;32:259-261.

2 Q X: Report to the third national oral health epidemiology investigation. Beijing: People's Medical Publishing House 2008

3 Bascones-Martinez A, Munoz-Corcuera M, Noronha S, Mota P, Bascones-Ilundain C, Campo-Trapero J: Host defence mechanisms against bacterial aggression in periodontal disease: Basic mechanisms. Med Oral Patol Oral Cir Bucal 2009;14:e680-685.

4 Giannobile WV: Salivary diagnostics for periodontal diseases. J Am Dent Assoc 2012;143:6S-11S.
5 Arigbede AO, Babatope BO, Bamidele MK: Periodontitis and systemic diseases: A literature review. J Indian Soc of Periodontol 2012;16:487-491.
6 Kim J, Amar S: Periodontal disease and systemic conditions: A bidirectional relationship. Odontology 2006;94:10-21.
7 Mawardi HH, Elbadawi LS, Sonis ST: Current understanding of the relationship between periodontal and systemic diseases. Saudi Med J 2015;36:150-158.
8 Soroye M, Ayanbadejo P, Savage K, Oluwole A: Association between periodontal disease and pregnancy outcomes. Odontostomatol Trop 2015;38:5-16.
9 Kudo C, Shin WS, Minabe M, Harai K, Kato K, Seino H, Goke E, Sasaki N, Fujino T, Kuribayashi N, Pearce YO, Taira M, Maeda H, Takashiba S, Periodontitis, Atherosclerosis P-T, Chiba C: Analysis of the relationship between periodontal disease and atherosclerosis within a local clinical system: A cross-sectional observational pilot study. Odontology 2015;103:314-321.
10 Pranckeviciene A, Siudikiene J, Ostrauskas R, Machiulskiene V: Severity of periodontal disease in adult patients with diabetes mellitus in relation to the type of diabetes. Biomed Pap Med Fac Univ Palacky Olomouc Czech Repub 2014;158:117-123.
11 Freudenheim JL, Genco RJ, LaMonte MJ, Millen AE, Hovey KM, Mai X, Nwizu N, Andrews CA, Wactawski-Wende J: Periodontal disease and breast cancer: Prospective cohort study of postmenopausal women. Cancer Epidemiol Biomarkers Prev 2016;25:43-50.
12 Jacob JA: Study links periodontal disease bacteria to pancreatic cancer risk. Jama 2016;315:2653-2654.
13 Chang JS, Tsai CR, Chen LT, Shan YS: Investigating the association between periodontal disease and risk of pancreatic cancer. Pancreas 2016;45:134-141.
14 Choi IA, Kim JH, Kim YM, Lee JY, Kim KH, Lee EY, Lee EB, Lee YM, Song YW: Periodontitis is associated with rheumatoid arthritis: A study with longstanding rheumatoid arthritis patients in korea. Korean J Intern Med 2016;31:977-986.
15 Benedyk M, Mydel PM, Delaleu N, Plaza K, Gawron K, Milewska A, Maresz K, Koziel J, Pyrc K, Potempa J: Gingipains: Critical factors in the development of aspiration pneumonia caused by porphyromonas gingivalis. J Innate Immun 2016;8:185-198.
16 Penova-Veselinovic B, Keelan JA, Wang CA, Newnham JP, Pennell CE: Changes in inflammatory mediators in gingival crevicular fluid following periodontal disease treatment in pregnancy: Relationship to adverse pregnancy outcome. J Reprod Immunol 2015;112:1-10.
17 Jeffcoat MK, Jeffcoat RL, Gladowski PA, Bramson JB, Blum JJ: Impact of periodontal therapy on general health: Evidence from insurance data for five systemic conditions. Am J Prev Med 2014;47:166-174.
18 Wang TF, Jen IA, Chou C, Lei YP: Effects of periodontal therapy on metabolic control in patients with type 2 diabetes mellitus and periodontal disease: A meta-analysis. Medicine 2014;93:e292.
19 Stanko P, Izakovicova Holla L: Bidirectional association between diabetes mellitus and inflammatory periodontal disease. A review. Biomed Pap Med Fac Univ Palacky Olomouc Czech Repub 2014;158:35-38.
20 Li X, Kolltveit KM, Tronstad L, Olsen I: Systemic diseases caused by oral infection. Clin Microbiol Rev 2000;13:547-558.
21 Moura IC, Benhamou M, Launay P, Vrtovsnik F, Blank U, Monteiro RC: The glomerular response to iga deposition in iga nephropathy. Semin Nephrol 2008;28:88-95.
22 Zhang L, Kong D, Meng H, Han C, Zhu J, Qiao J, He Y, Wang T, Li X, Zhang F, Jin X: Plasma gelsolin promotes proliferation of mesangial cell in iga nephropathy. Cell Physiol Biochem 2016;40:1473-1486.
23 Tan M, Li W, Zou G, Zhang C, Fang J: Clinicopathological features and outcomes of iga nephropathy with hematuria and/or minimal proteinuria. Kidney Blood Press Res 2015;40:200-206.
24 De Angelis M, Montemurno E, Piccolo M, Vannini L, Lauriero G, Maranzano V, Gozzi G, Serrazanetti D, Dalfino G, Gobbetti M, Gesualdo L: Microbiota and metabolome associated with immunoglobulin a nephropathy (igan). PloS one 2014;9:e99006.
25 Cao YL, Qiao M, Xu ZH, Zou GM, Ma LL, Li WG, Xu BH: [the clinical study of iga nephropathy with severe chronic periodontitis and aggressive periodontitis]. Zhonghua yi xue za zhi 2016;96:9-13.
26 Benjamini Y, Hochberg Y: Controlling the false discovery rate: A practical and powerful approach to multiple testing. J R Stat Soc Series B Stat Methodol (Methodological) 1995;57:289-300.

27 Singhrao SK, Harding A, Poole S, Kesavalu L, Crean S: Porphyromonas gingivalis periodontal infection and its putative links with alzheimer's disease. Mediators Inflamm 2015;2015:137357.
28 AlJehani YA: Risk factors of periodontal disease: Review of the literature. Int J Dent 2014;2014:182513.
29 Griffen AL, Beall CJ, Campbell JH, Firestone ND, Kumar PS, Yang ZK, Podar M, Leys EJ: Distinct and complex bacterial profiles in human periodontitis and health revealed by 16s pyrosequencing. The ISME journal 2012;6:1176-1185.
30 Abusleme L, Dupuy AK, Dutzan N, Silva N, Burleson JA, Strausbaugh LD, Gamonal J, Diaz PI: The subgingival microbiome in health and periodontitis and its relationship with community biomass and inflammation. The ISME journal 2013;7:1016-1025.
31 Imai H, Fujita T, Kajiya M, Ouhara K, Yoshimoto T, Matsuda S, Takeda K, Kurihara H: Mobilization of tlr4 into lipid rafts by aggregatibacter actinomycetemcomitans in gingival epithelial cells. Cell Physiol Biochem 2016;39:1777-1786.
32 Tomana M, Novak J, Julian BA, Matousovic K, Konecny K, Mestecky J: Circulating immune complexes in iga nephropathy consist of iga1 with galactose-deficient hinge region and antiglycan antibodies. J Clin Invest 1999;104:73-81.
33 Piccolo M, De Angelis M, Lauriero G, Montemurno E, Di Cagno R, Gesualdo L, Gobbetti M: Salivary microbiota associated with immunoglobulin a nephropathy. Microb Ecol 2015;70:557-565.
34 Dashper SG, Seers CA, Tan KH, Reynolds EC: Virulence factors of the oral spirochete treponema denticola. J Dent Res 2011;90:691-703.
35 Sela MN: Role of treponema denticola in periodontal diseases. Crit Rev Oral Biol Med 2001;12:399-413.
36 Sarkar J, McHardy IH, Simanian EJ, Shi W, Lux R: Transcriptional responses of treponema denticola to other oral bacterial species. PloS one 2014;9:e88361.
37 Gmur R, Thurnheer T: Direct quantitative differentiation between prevotella intermedia and prevotella nigrescens in clinical specimens. Microbiology 2002;148:1379-1387.
38 Kononen E: Pigmented prevotella species in the periodontally healthy oral cavity. FEMS Immunol Med Microbiol 1993;6:201-205.
39 Han YW, Ikegami A, Bissada NF, Herbst M, Redline RW, Ashmead GG: Transmission of an uncultivated bergeyella strain from the oral cavity to amniotic fluid in a case of preterm birth. J Clin Microbiol 2006;44:1475-1483.
40 Colombo AP, Bennet S, Cotton SL, Goodson JM, Kent R, Haffajee AD, Socransky SS, Hasturk H, Van Dyke TE, Dewhirst FE, Paster BJ: Impact of periodontal therapy on the subgingival microbiota of severe periodontitis: Comparison between good responders and individuals with refractory periodontitis using the human oral microbe identification microarray. J Periodontol 2012;83:1279-1287.
41 Vielkind P, Jentsch H, Eschrich K, Rodloff AC, Stingu CS: Prevalence of actinomyces spp. In patients with chronic periodontitis. Int J Med Microbiol 2015;305:682-688.
42 Souto R, Andrade AF, Uzeda M, Colombo APV: Prevalence of "non-oral" pathogenic bacteria in subgingival biofilm of subjects with chronic periodontitis. Braz J Microbiol 2006;37:208-215.
43 Dyer JK, Reinhardt RA, Petro TM, Strom EA: Serum antibody responses in human periodontitis to cellular components of capnocytophaga. Arch Oral Biol 1992;37:725-731.
44 Jolivet-Gougeon A, Sixou JL, Tamanai-Shacoori Z, Bonnaure-Mallet M: Antimicrobial treatment of capnocytophaga infections. Int J Antimicrob Agents 2007;29:367-373.
45 Bhatia M, Urolagin SS, Pentyala KB, Urolagin SB, K BM, Bhoi S: Novel therapeutic approach for the treatment of periodontitis by curcumin. J Clin Diagn Res 2014;8:ZC65-69.
46 Pudakalkatti PSBA, Hattarki SA, Kambali SS, Naik RM: Detection and prevalence of capnocytophaga in periodontal health and disease. J Orofac Sci 2016;8:92-95.
47 Huang S, Yang F, Zeng X, Chen J, Li R, Wen T, Li C, Wei W, Liu J, Chen L, Davis C, Xu J: Preliminary characterization of the oral microbiota of chinese adults with and without gingivitis. BMC Oral Health 2011;11:33.
48 Cho SB, Zheng Z, Ahn KJ, Choi MJ, Cho S, Kim DY, Lee HS, Bang D: Serum iga reactivity against groel of streptococcus sanguinis and human heterogeneous nuclear ribonucleoprotein a2/b1 in patients with behcet disease. Br J Dermatol 2013;168:977-983.
49 Schmitt R, Carlsson F, Morgelin M, Tati R, Lindahl G, Karpman D: Tissue deposits of iga-binding streptococcal m proteins in iga nephropathy and henoch-schonlein purpura. Am J Pathol 2010;176:608-618.