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Subgingival microbiome in Chinese patients with generalized aggressive periodontitis compared to healthy controls

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ABSTRACT

Objective: The aim of the study was to profile the subgingival microbiome of Chinese adults with generalized aggressive periodontitis (GAgP) using human oral microbe identification microarray (HOMIM), and to compare the results with matched periodontal healthy controls.

Design: 15 subjects with GAgP and 15 age- and gender- matched periodontal healthy controls were included. Subgingival plaque samples were collected from the deepest pockets of patients with GAgP and matched sites in controls and then analyzed by 16S rRNA-based microarrays. Student's paired *t*-test was used to compare clinical parameters and mean number of bacterial taxa detected between the two groups. Fisher's exact probability test and Wilcoxon Rank Sum were used to compare bacterial species between all samples. A multiple linear regression model was used for correlations among age, gender and bacterial with clinical parameters.

Results: From a total sum of 379 strains tested, 171 bacterial strains were detected from subgingival plaques of the GAgP patients, more than the 157 strains detected in control group. Mean number of subgingival bacterial taxa detected in GAgP group was 68 (SD = 21.06) while in control group was 45 (SD = 21.60). 47 bacterial taxa were detected more frequently in GAgP group while 12 taxa were more prevalent in control group. The significantly more prevalent and abundant taxa of bacteria in GAgP group included *Filifactor alocis*, *Desulfobulbus* sp., *Fretibacterium* sp., *Porphyromonas gingivalis*, *Tannerella forsythia*, *Porphyromon as endodontalis*, *Peptostreptococcaceae* spp., *Parvimonas micra*, *Eubacterium nodatum* and *Eubacterium saphenum*. Meanwhile the more abundant taxa in control group were *Streptococcus spp*. and *Pseudomonas aeruginosa*.

Conclusions: There are more taxa of bacteria in subgingival plaques of Chinese patients with GAgP than in healthy controls. *F. alocis*, *Desulfobulbus* sp., *Fretibacterium* sp., *P. gingivalis* and *T. forsythia* are strongly associated with GAgP. High-throughout microbiological results may help dentists have a better understanding of subgingival microbiome of GAgP.

1. Introduction

Generalized aggressive periodontitis (GAgP) usually occurs in individuals younger than 35-year old. This disease is characterized by a relatively rapid destruction of periodontal tissue in otherwise systemically healthy individuals (Lang et al., 1999; Teughels, Dhondt, Dekeyser, & Quirynen, 2014). Patients with GAgP have generalized interproximal attachment loss affecting at least three permanent teeth other than first molars and incisors (Lang et al., 1999). Thus, GAgP may result in severe consequences if without proper periodontal treatment.

Initiation and progression of GAgP require simultaneous occurrence of a number of factors, such as microbial challenge, genetic and acquired risk factors (Page & Kornman, 1997). Among them, bacterial plaque has been suggested as the initiating factor of periodontitis (Socransky & Haffajee, 1994; Teles, Teles, Frias-Lopez, Paster, & Haffajee, 2013). Studies have shown that certain species of bacteria were strongly linked to GAgP. It has also been indicated that *Aggregatibacter actinomycetemcomitans* (*Aa*) is an important pathogen of this disease (Cortelli, Cortelli, Jordan, Haraszthy, & Zambon, 2005; Darby, Hodge, Riggio, & Kinane, 2000; Slots, Feik, & Rams, 1990), although it may vary among subjects of different races or ethnic groups (Celenligil & Ebersole, 1998; Takeuchi, Umeda, Ishizuka, Huang, & Ishikawa, 2003; Wang, Yang, & Shang, 1997). Other studies reported that the prevalence and abundance of other periodontal pathogens,

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such as *Porphyromonas gingivalis* (*Pg*), *Tannerella forsythia* (*Tf*), *Prevotella intermedia* (*Pi*), *Peptostreptococcus micros*, *Fusobacterium* species, *Selenomonas sputigena* and spirochaetes (Darby et al., 2000; Kamma, Nakou, & Manti, 1994; Lopez, 2000; Ximenez-Fyvie et al., 2006) were also increased in GAgP patients.

However, previous studies on pathogens of GAgP were heterogeneous in the aspect of research methods and most of them only focused on limited common periodontal pathogens. Proper characterization of the oral microbiome is dependent upon the technologies used to determine the relative proportions of specific bacterial species in various oral sites. Human Oral Microbe Identification Microarray (HOMIM), using 16S ribosomal RNA (rRNA) gene sequences for species identification, for the first time, provided 379 species-level probes capable of identifying 293 predominant oral bacterial species. Over 700 bacterial species have been detected in the oral cavity by using this new method, among which only about 50% have been cultivated (Aas, Paster, Stokes, Olsen, & Dewhirst, 2005; Paster et al., 2001). In China, few studies investigating on microbial profiles of different types of periodontitis have used high-throughput techniques.

One study using high-throughput techniques found that there was a kinship in the phylogenetic architecture of microbiota among Chinese AgP patients and their family members (Li et al., 2015). Also, there were studies focused on microbial community shifts of subgingival plaque in GAgP patients following non-surgical periodontal therapy (Han, Wang, & Ge, 2017; Liu et al., 2017). However, there is no study comparing the difference of subgingival microbial profile between Chinese GAgP patients and periodontal healthy subjects using high-throughput techniques.

The purpose of the present study was to compare 379 taxa of microbes in subgingival plaques of Chinese GAgP patients and matched periodontal healthy subjects using HOMIM, and to analyze the correlation between microbiological results and periodontal clinical parameters.

2. Material and methods

2.1. Participant selection

Fifteen Chinese patients with GAgP were recruited at the Department of Periodontology, Peking University School and Hospital of Stomatology, Beijing, China. Fifteen age- and gender- matched healthy controls were selected from graduate students and staffs of the Peking University School of Stomatology (PKUSS). All participants were informed about the study protocols and signed an informed consent previously approved by the Institutional Review Board at Peking University, School of Stomatology (PKUSSIRB-2012048). The medical and dental histories were taken from all participants. GAgP was diagnosed according to the criteria of the American Academy of Periodontology (Lang et al., 1999) except familial aggregation. It is difficult to retrieve reliable information on familial aggregation from patients, so familial aggregation was not in included in our inclusion criteria. In addition, patients had at least 20 teeth (excluding third molars) and at least four sites on different quadrants (three of them other than central incisors or first molars) with a probing pocket depth (PPD) ≥ 5 mm, clinical attachment loss (CAL) ≥ 2 mm and bleeding on probing (BOP). Periodontal healthy (PH) was defined by the presence of mean PPD ≤ 3 mm, CAL ≤ 1 mm and sites with bleeding index (BI) ≥ 2 were less than 10%. The Bleeding Index (BI) (Mazza, Newman, & Sims, 1981) was graded on a scale from 0 to 5:

0 = normal appearing, healthy gingiva;
1 = colour changes related to inflammation but no bleeding;
2 = slight bleeding that remains at the point of probing;
3 = bleeding extending from the point of probing and flowing around the gingival margin;
4 = profuse bleeding that overflows the gingival margin;
5 = spontaneous bleeding.

Table 1
Demographic and clinical characteristics of participants (mean ± standard deviation).

	GAgP ($N = 15$)	Control group ($N = 15$)	p-Value
Mean age (yrs ± SD)	27.73 ± 3.01	27.73 ± 3.01	–
Gender (male/female)	7/8	7/8	–
Clinical parameters at all sites			
PPD (mm ± SD)	5.00 ± 0.82	2.07 ± 0.17	< 0.001*
CAL (mm ± SD)	4.87 ± 1.02	0.01 ± 0.02	< 0.001*
BI (± SD)	3.57 ± 0.66	0.68 ± 0.43	< 0.001*
PLI (± SD)	1.24 ± 0.59	0.15 ± 0.09	< 0.001*
Clinical parameters at sampled sites			
PPD (mm ± SD)	7.53 ± 0.92	2.47 ± 0.36	< 0.001*
CAL(mm ± SD)	7.13 ± 1.48	0.00 ± 0.00	< 0.001*
BI (± SD)	3.63 ± 0.71	0.50 ± 0.45	< 0.001*
PLI (± SD)	1.03 ± 0.60	0.13 ± 0.13	< 0.001*

*$p < 0.01$: Paired t-test.
SD: standard deviation.
GAgP: generalized aggressive periodontitis.
PPD: probing pocket depth.
CAL: clinical attachment loss.
BI: bleeding index.
PLI: plaque index.

Exclusion criteria for both groups included systemic diseases or conditions that could influence the progression and/or clinical characteristics of periodontal disease, periodontal treatment or antibiotics within the preceding 3 months, smokers, and pregnant/lactating women.

2.2. Clinical measurements

The following periodontal clinical parameters were measured by one calibrated examiner at the initial visit for all patients: PPD, CAL (distance from the CEJ to the bottom of the pocket), BI (Mazza et al., 1981) and plaque index (PLI) (Löe, 1967). The PLI was graded on a scale from 0 to 3:

0 = no plaque;
1 = a film of plaque adhering to the free gingival margin and adjacent area of the tooth, which cannot be seen with the naked eye. But only by using disclosing solution or by using probe;
2 = moderate accumulation of deposits within the gingival pocket, on the gingival margin and/or adjacent tooth surface, which can be seen with the naked eye;
3 = abundance of soft matter within the gingival pocket and/or on the tooth and gingival margin.

Measurements were performed with a Williams periodontal probe (Hu-Friedy Mfg.Co., LLC, Chicago, IL, USA) at 6 sites per tooth and were recorded. Periapical X-rays were taken to confirm diagnosis of GAgP. Intra-examiner calibration was obtained with 80% of duplicate measures of probing depth and CAL within 1 mm.

2.3. Collection of subgingival plaque samples

One week after initial visit, subgingival plaque samples were obtained from the mesio-buccal sites (two sites each quadrant) demonstrating deepest PPD (PPD ≥ 5 mm and CAL ≥ 3 mm). The eight samples were pooled into a single sample tube. Pooled subgingival plaque samples of PH controls were collected from sites matched with the patients. The area of collection was isolated with cotton rolls, and supragingival plaque was carefully removed. Subgingival plaque samples were collected by inserting a sterile endodontic paper-point into the

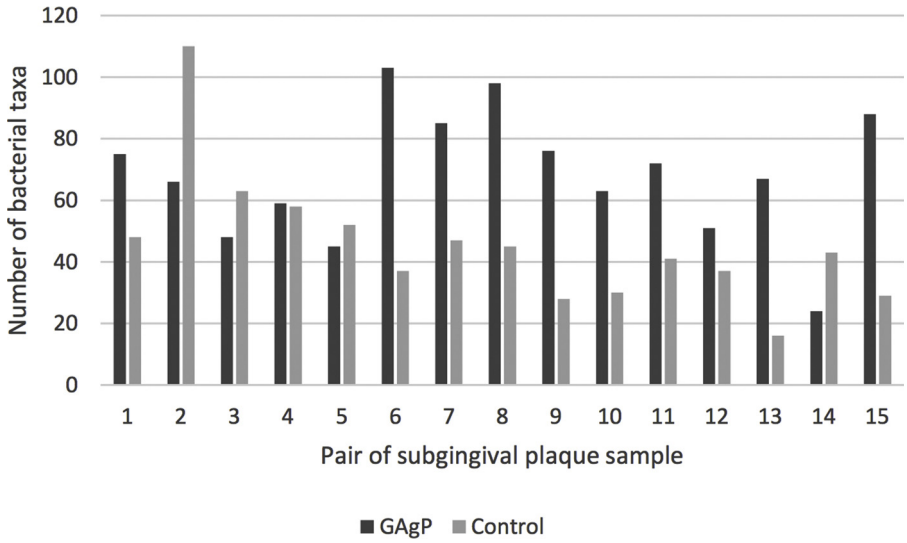

Fig. 1. Number of subgingival bacterial taxa detected in each paired sample. Black: GAgP group, grey: control group.

periodontal pockets for 30 s. Samples were then placed in 1.5-mL Ep tubes and stored at −80 °C until processed.

2.4. DNA isolation and microarray analysis

Bacteria were separated from the paper-points by adding 100 μL of phosphate-buffered saline (PBS) to the tubes, followed by vortexing. The paper-points were then removed, and DNA was isolated with a TIANamp Micro DNA Kit (Tiangen Biotech, Beijing, CN) using the microtissue protocol according to the manufacturer's instructions. A 40 μL volume of each sample with a minimum DNA concentration of 20 ng/μL was obtained. After lyophilization, DNA samples were submitted to the Forsyth Institute (Cambridge MA, USA) for HOMIM analysis. Briefly, 16S rRNA genes were PCR-amplified from DNA extracts with 16S rRNA universal forward and reverse primers and labelled via incorporation of Cy3-dCTP in a second nested PCR. HOMIM used 16S rRNA-based, reverse-capture oligonucleotide probes, which were printed on aldehyde-coated glass slides and probed with labelled PCR products (described above) which were hybridized in duplicate. The microarray slides were scanned in an Axon 4000B scanner, and crude data were extracted with GenePix Pro software (Molecular Devices, Sunnyvale, CA, USA). Microbial profiles were generated from image files of scanned arrays with a HOMIM online analysis tool (http://bioinformatics.forsyth.org/homim/). Detection of a particular taxon was determined by the presence of a fluorescent spot for that unique probe. Blank buffer control, negative control and positive control (16S Universal E29) were sequenced at the same time. A mean intensity for each taxon was calculated from hybridization spots of the same probe, and signals were normalized by comparison of individual signal intensities with the average of signals from universal probes. Any original signal that was less than two times the background value was re-set to 1 and was assigned to the signal abundance 0. Signals greater than 1 were categorized into scores from 1 to 5, corresponding to ranked signal abundances.

2.5. Statistical analysis

A student's paired *t*-test was applied for differences in clinical parameters and mean number of subgingival bacterial taxa detected between the two groups. Fisher's exact probability test was performed to compare prevalence of each taxon between GAgP and control group. Wilcoxon Rank Sum test with Benjamini-Hochberg adjustment was used to compare abundance of taxa between two groups. Principal component analysis (PCA) was used to demonstrate the maximum variation between all samples. A multiple linear regression model was used for correlations among age, gender and bacterial with clinical parameters. Pearson correlation analysis was used for correlations between gender and bacterial taxa in GAgP group. SPSS Statistics 20.0 (SPSS Inc., Chicago, IL, USA) was used for statistical analyses of clinical data. TIGR MultiExperiment Viewer (Dana-Farber Cancer Institute, Boston, MA, USA) was used for non-parametric statistical analysis. A p-value of < 0.05 was considered statistically significant.

3. Results

3.1. General information and periodontal status of participants

Fifteen patients with GAgP and fifteen healthy controls were matched in age, gender and sampled sites. All clinical parameters were significantly different between GAgP and control group as expected, while demographic characteristics were not different (Table 1).

3.2. Prevalence of subgingival microbiome

In total, 379 bacterial taxa were analyzed in the subgingival plaque samples of all individuals. All samples collected were successfully analyzed. 171 taxa were detected in GAgP group and 157 taxa were detected in periodontal healthy control group. Mean number of subgingival bacterial taxa detected in GAgP group was 68 (standard deviation, SD = 21.06) while in control group was 45 (SD = 21.60). Number of subgingival bacterial taxa in GAgP group was statistically higher than in control group ($p = 0.019$). Fig. 1 showed the number of subgingival bacterial taxa detected in each paired sample in each group.

Among them, 59 bacterial taxa showed significant differences between groups for prevalence. 47 taxa were more prevalent in subjects with GAgP while other 12 taxa were more prevalent in healthy controls (Fig. 2). In the 59 bacterial taxa, 21 taxa were only detected in GAgP group (Fig. 2, taxa marked with #). Further analysis showed that among the 21 taxa, the prevalence of 1 taxon (*Peptostreptococcaceae* spp.) was more than 80%; 6 taxa presented in more than 60% samples and other 2 taxa presented in approximately 60% samples. There was 1 taxon only detected in control group (*Streptococcus cristatus* HOT-578_AA47) (Fig. 2, taxon marked with ※). 2 taxa were detected simultaneously in 14 patients with GAgP and 1 periodontal healthy control (*Filifactor alocis* HOT-539_AA69 and *Desulfobulbus* sp. HOT-041_K70).

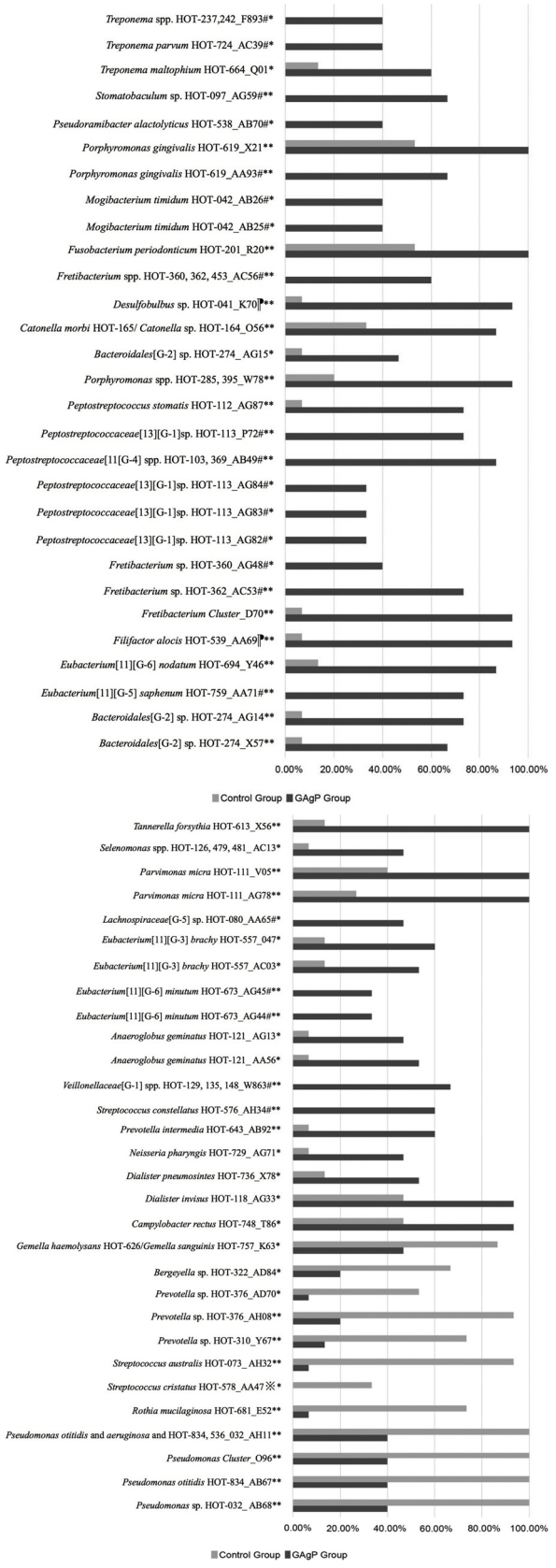

Fig. 2. Prevalence of Taxa between GAgP and control groups (only taxa with significant difference are shown). Taxa marked with # were only detected in GAgP group. Taxon marked with ※ was only detected in control group. Taxa marked with □ were detected simultaneously in 14 patients with GAgP and 1 periodontal healthy control. *$p < 0.05$, **$p < 0.01$: Fisher's exact probability test.

Table 2
Top 20 most abundant bacterial taxa detected in GAgP and control group.

Group	Top 20 most abundant bacterial taxa detected
GAgP	
	*Fusobacterium Cluster*_AE01
	Filifactor alocis HOT-539_AA69
	Tannerella forsythia HOT-613_X56
	Parvimonas micra HOT-111_V05
	Streptococcus constellatus HOT-576/*Streptococcus intermedius* HOT-644_F48
	Campylobacter concisus HOT-575/*Campylobacter rectus* HOT-748_X36
	Parvimonas micra HOT-111_AG78
	Desulfobulbus sp. HOT-041_K70
	Porphyromonas gingivalis HOT-619_X21
	Eubacterium[11][G-7] *yurii* HOT-377/*Peptostreptococcaceae*[11][G-7] sp. HOT-106_W84
	Streptococcus constellatus HOT-576/*Streptococcus intermedius* HOT-644_AB77
	Eubacterium[11][G-6] *nodatum* HOT-694_Y46
	Fusobacterium periodonticum HOT-201_R20
	Porphyromonas endodontalis HOT-273/*Porphyromonas* spp. HOT-285,395_W78
	Eubacterium[11][G-5] *saphenum* HOT-759_AA71
	Campylobacter concisus HOT-575/*Campylobacter rectus* HOT-748_T86
	Haemophilus parainfluenzae HOT-718_W79
	Campylobacter curvus HOT-580/*Campylobacter rectus* HOT-748/ *Campylobacter showae* HOT-763_X37
	Streptococcus oralis HOT-707/*Streptococcus* sp. HOT-064_F46
	Campylobacter gracilis HOT-623_Q04
Control	
	Pseudomonas Cluster_O96
	Pseudomonas aeruginosa HOT-536/*Pseudomonas otitidis* HOT-834_AB67
	Pseudomonas aeruginosa HOT-536/*Pseudomonas otitidis* HOT-834/ *Pseudomonas* sp. HOT-032_AB68
	Streptococcus oralis HOT-707/*Streptococcus* sp. HOT-064_F46
	Pseudomonas otitidis and *aeruginosa* and HOT-834,536_032_AH11
	Haemophilus parainfluenzae HOT-718_W79
	Gemella haemolysans HOT-626_AG51
	Streptococcus australis HOT-073_AH32
	Fusobacterium Cluster_AE01
	Streptococcus constellatus HOT-576/*Streptococcus intermedius* HOT-644_F48
	Gemella haemolysans HOT-626/*Gemella sanguinis* HOT-757_K63
	Campylobacter concisus HOT-575/*Campylobacter rectus* HOT-748_X36
	Prevotella sp. HOT-317_AH08
	Neisseria elongata HOT-598_AA75
	Campylobacter showae HOT-763_X35
	Capnocytophaga granulosa HOT-325_AG23
	Eubacterium[11][G-7] *yurii* HOT-377/*Peptostreptococcaceae*[11][G-7] sp. HOT-106_W84
	Campylobacter showae HOT-763_W36
	Actinomyces georgiae HOT-617_AG01
	Rothia dentocariosa HOT-587/*Rothia mucilaginosa* HOT-681_E52

3.3. Abundance of subgingival microbiome

The top 20 most abundant taxa detected in GAgP group and control group were listed in Table 2. A total number of 17 taxa showed significantly difference in abundance between GAgP and control group. Subjects with GAgP demonstrated significantly higher abundance of 12 taxa, while periodontal healthy controls demonstrated significantly higher abundance of 5 taxa (Table 3). Among the significantly higher abundant 12 taxa in GAgP group, 3 taxa were only detected in GAgP group and had prevalence approximately of 60%-80% (*Eubacterium* HOT-759_AA71, *Peptostreptoxoccaceae* HOT-113_P72 and *Peptostreptococcaceae* spp. HOT-103, 369_AB49); the rest of 9 taxa had prevalence approximately of 80–100%.

3.4. Principal component analysis (PCA)

Subgingival plaque compositions of subjects with GAgP and healthy controls were separated according to three-dimensional PCA (Fig. 3).

Table 3
Abundance of taxa with significant difference between GAgP and control group.

Group	Bacterial with significant difference	W-value	*p*-Value[a]
GAgP			
	Eubacterium[11][G-5] *saphenum* HOT-759_AA71	3.97	< 0.001
	Peptostreptococcaceae[13][G-1] sp. HOT-113_P72	3.97	< 0.001
	Parvimonas micra HOT-111_V05	3.98	< 0.001
	Fretibacterium sp. HOT-359/*Fretibacterium sp.* HOT-362_AC53	3.99	< 0.001
	Porphyromonas endodontalis HOT-273/*Porphyromonas* spp. HOT-285,395_W78	4.08	< 0.001
	Eubacterium[11][G-6] *nodatum* HOT-694_Y46	4.17	< 0.001
	Filifactor alocis HOT-539_AA69	4.36	< 0.001
	Porphyromonas gingivalis HOT-619_X21	4.37	< 0.001
	Desulfobulbus sp. HOT-041_K70	4.60	< 0.001
	Peptostreptococcaceae[11][G-4] spp. HOT-103,369_AB49	4.61	< 0.001
	Fretibacterium Cluster_D70	4.71	< 0.001
	Tannerella forsythia HOT-613_X56	4.90	< 0.001
Control			
	Streptococcus australis HOT-073_AH32	−4.59	< 0.001
	Streptococcus oralis HOT-707/*Streptococcus* sp. HOT-064_F46	−4.57	< 0.001
	Pseudomonas aeruginosa HOT-536/*Pseudomonas otitidis* HOT-834_AB67	−4.41	< 0.001
	Pseudomonas aeruginosa HOT-536/*Pseudomonas otitidis* HOT-834/*Pseudomonas* sp. HOT-032_AB68	−4.35	< 0.001
	Pseudomonas otitidis and *aeruginosa* and HOT-834,536_032_AH11	−4.00	< 0.001

Only taxa with significant difference ($p < 0.05$) are shown.
[a]Non-parametric analysis (Wilcoxon Rank sum test), $p < 0.001$ corrected using Benjamini-Hochberg adjustment.

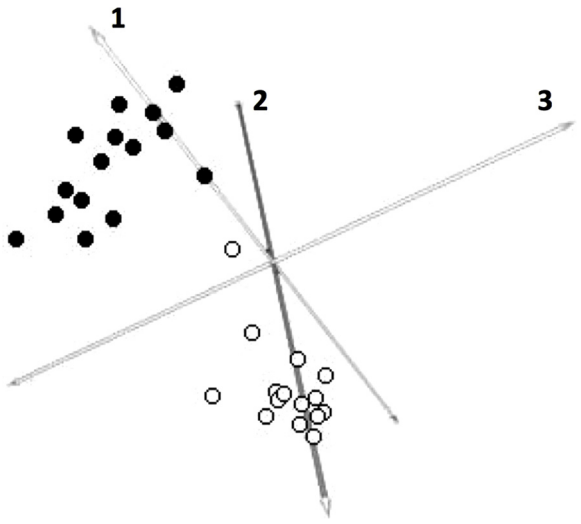

Fig. 3. Principal Component Analysis of Subgingival Plaques. Black dots: GAgP group, white dots: control group. Variance of axis 1: 33.73%, variance of axis 2: 15.92%, variance of axis 3: 6.02%.

The variances of the top three principal components were as follows: 33.726%, 15.924%, and 06.017%. However, there was no clear-cut demarcation line between two groups.

3.5. Relationship between subgingival microbiome and clinic parameters

A multiple linear regression model demonstrated that gender and several bacteria were correlated to periodontal clinical parameters (Table 4). Male subjects had deeper PPD at sampled sites than female subjects.

Abundance of *P. gingivalis* HOT-619_X21 showed a positive correlation with PPD, CAL and BI, respectively, at all sites. Abundance of *Eubacterium* [G-5] *saphenum* HOT-759_AA71 was positively correlated to mean PPD at sample sites. Abundance of *Pseudomonas otitidis* and *aeruginosa*, and HOT-834, 536_032_AH11 was positively correlated to mean BI at all sites.

Abundance of *P. aeruginosa* HOT-536 showed negative correlation with mean PPD, CAL and BI at all sites. Abundance of *Fretibacterium* sp. HOT-359 and *Eubacterium*[11][G-6] *nodatum* HOT-694_Y46 were negatively correlated to mean BI at all sites.

3.6. Relationship between subgingival microbiome and gender in GAgP group

In GAgP group, Pearson correlation coefficient demonstrated that gender and several bacteria were correlated (Table 5). Sixteen bacterial taxa, including *Peptostreptococcus stomatis* HOT-112_AG87, *Propionibacterium propionicum* HOT-739_AB72, TM7[G-5] spp. HOT-356,437_O32, *Bacteroidetes*[G-3] spp. HOT-281,365_AG17, *Mogibacterium timidum* HOT-042_AB25, *M. timidum* HOT-042_AB26, *Streptococcus anginosus* HOT-543/*Streptococcus gordonii* HOT-622_F49, *Campylobacter concisus* HOT-575_O46, *Dialister pneumosintes* HOT-736_AG34, *D. pneumosintes* HOT-736_X78, *Streptococcus constellatus* HOT-576/*Streptococcus intermedius* HOT-644_AB77, *Lachnospiraceae*[G-3] sp. HOT-100_AG58, *Lachnospiraceae*[G-5] sp. HOT-080_AA65, *Selenomonas infelix* HOT-639/*Selenomonas* spp. HOT-126,479,481_AC13, *S. infelix* HOT-639/*Selenomonas* spp. HOT-126,479,481_O54, and *Selenomonas* spp. HOT-138,146,892_Q52 were correlated with male patients. Only two taxa, *P. gingivalis* HOT-619_AA93 and *P. gingivalis* HOT-619_X21 were correlated with female patients. No correlation was found between microbiome and gender in control group.

4. Discussion

More than 400 of bacterial species have been commonly detected in the subgingival environment (Paster et al., 2001). The subgingival microbiome of GAgP has been examined by either culturing or molecular methods targeting limited common periodontal pathogens (Casarin et al., 2010; Castillo et al., 2011; Tomita et al., 2013). To our knowledge, the present study represents the first report using HOMIM to detect and to compare subgingival plaque bacteria between GAgP and matched periodontal healthy controls in a Chinese population.

In the present study, we tested 379 bacterial strains in subgingival plaques of 15 Chinese GAgP patients and 15 matched periodontal healthy subjects. There were more bacterial strains in GAgP group (171) than in healthy control group (157). It is well known that there is a difference in the microbes between GAgP and the control group, but it is not clear what kind of difference exists. It is still not known whether there are differences in the species of the bacterial flora or changes in the ratio of bacterial species. In our study, it was demonstrated that more taxa were in GAgP than in healthy controls. The results of principal component analysis further confirmed that the microbial characteristics of GAgP patients and periodontally healthy controls were different and subgingival microbiome analysis alone could distinguish the two groups of people, which can be a strong evidence for the differences of bacterial species between the two groups. Although there were studies showed the bacterial numbers associated with periodontal disease are up to 10 times larger than those associated with health (Lovegrove, 2004), most previous studies mainly focused on the limited number of known periodontal pathogenic bacteria. Our study extended the numbers of bacterial strains tested in GAgP patients and periodontal healthy controls by using HOMIM. However, from the view of ecology of microbial communities, high diversity of bacterial taxa has been generally associated with health (Turnbaugh et al., 2007), and temporal stability (Flores et al., 2014), which was contrary to our findings. The results of our study indicated there may be negative relationship between the number of subgingival bacterial strains and periodontal health. Future studies will be needed to disclose more information.

Table 4
Correlation between gender, bacterial taxa and clinical parameters.

Dependent variable	Independent variable	Standardized coefficients	*p*-Value
sPPD			
	Gender[a,*]	−0.175	0.029*
	Eubacterium[11][G-5] *saphenum* HOT-759_AA71	0.496	0.047*
sBI			
	Fretibacterium sp. HOT-359	−0.932	0.041*
mPPD			
	Pseudomonas aeruginosa HOT-536	−2.009	0.019*
	Porphyromonas gingivalis HOT-619_X21	0.650	0.023*
mCAL			
	Pseudomonas aeruginosa HOT-536	−1.461	0.008**
	Porphyromonas gingivalis HOT-619_X21	0.465	0.012*
mBI			
	Pseudomonas aeruginosa HOT-536	−2.377	0.012*
	Pseudomonas otitidis and *aeruginosa* and HOT-834,536_032_AH11	1.805	0.027*
	Fretibacterium sp. HOT-359	−0.896	0.035*
	Eubacterium[11][G-6] *nodatum* HOT-694_Y46	−0.504	0.043*
	Porphyromonas gingivalis HOT-619_X21	0.685	0.027*

Only data with significant difference ($p < 0.05$) are shown.
*$p < 0.05$.
**$p < 0.01$: Multiple linear regression.
[a]Gender: Male = 1, Female = 2.
sPPD: mean PPD at sampled sites.
sBI: mean BI at sampled sites.
mPPD: mean PPD at all sites.
mCAL: mean CAL at all sites.
mBI: mean BI at all sites.

Table 5
Correlation between gender and bacterial taxa in GAgP group.

Group	Bacterial taxa	Pearson correlation coefficient	*p*-Value
GAgP			
	Peptostreptococcus stomatis HOT-112_AG87	−0.563	0.029*
	Propionibacterium propionicum HOT-739_AB72	−0.521	0.046*
	TM7[G-5] spp. HOT-356,437_O32	−0.535	0.040*
	Bacteroidetes[G-3] spp. HOT-281,365_AG17	−0.543	0.037*
	Mogibacterium timidum HOT-042_AB25	−0.518	0.048*
	Mogibacterium timidum HOT-042_AB26	−0.562	0.029*
	Porphyromonas gingivalis HOT-619_AA93	0.625	0.013*
	Porphyromonas gingivalis HOT-619_X21	0.543	0.037*
	Streptococcus anginosus HOT-543/*Streptococcus gordonii* HOT-622_F49	−0.533	0.041*
	Campylobacter concisus HOT-575_O46	−0.535	0.040*
	Dialister pneumosintes HOT-736_AG34	−0.808	0.000**
	Dialister pneumosintes HOT-736_X78	−0.785	0.001**
	Streptococcus constellatus HOT-576/*Streptococcus intermedius* HOT-644_AB77	−0.529	0.043*
	Lachnospiraceae[G-3] sp. HOT-100_AG58	−0.535	0.040*
	Lachnospiraceae[G-5] sp. HOT-080_AA65	−0.537	0.039*
	Selenomonas infelix HOT-639/*Selenomonas* spp. HOT-126,479,481_AC13	−0.774	0.001**
	Selenomonas infelix HOT-639/*Selenomonas* spp. HOT-126,479,481_O54	−0.754	0.001[3]
	Selenomonas spp. HOT-138,146,892_Q52	−0.635	0.011*

Gender: Male = 1, Female = 2.
Only data with significant difference ($p < 0.05$) are shown.
*$p < 0.05$.
**$p < 0.01$: Pearson correlation.

Our results also showed that periodontal clinical parameters were correlated with gender and abundance of certain subgingival bacteria taxa, which indicated that certain microbiological components of subgingival plaque may be associated with periodontal conditions in GAgP patients, and each gender may have different periodontal pathological bacteria.

It was found in this study that *P. gingivalis* HOT-619_X21 and *Eubacterium*[11][G-5] *saphenum* HOT-759_AA71 may have played a destructive role in GAgP in Chinese population. These two taxa had both high prevalence and abundance in GAgP group, significantly two-fold higher than the healthy control group. Similarly, the genus *Porphyromonas* was found to be significantly increased in the gingivitis patients in China in one of our recently studies (Deng, Ouyang, Chu, & Zhang, 2017). The present results were consistent with other studies that found the prevalence and abundance of *Porphyromonas* were higher in GAgP patients (Li et al., 2015; Liu et al., 2017). Moreover, the abundance of *P. gingivalis* HOT-619_X21 showed positive correlation with PPD, CAL and BI at all sites. The abundance of *Eubacterium*[11][G-5] *saphenum* HOT-759_AA71 showed positive correlation with PPD at sampled sites. However, another strain of *P. gingivalis* (HOT-619_AA93) did not show any correlation with the clinical parameters, even though it was only detected in the GAgP group. This may indicate that in

Chinese population, there might be a close relationship between the strain of *P. gingivalis* HOT-619_X21 and GAgP.

In previous studies, *F. alocis* (Fa), *Desulfobulbus* sp. and *Fretibacterium* sp. have been reported associated with chronic periodontitis and/or localized aggressive periodontitis (Kumar et al., 2006; Schlafer et al., 2010; Shaddox et al., 2012; Teles et al., 2011; You, Mo, Watt, & Leung, 2013). Our study was the first time reporting these species were of high prevalence and abundance in Chinese patients with GAgP. In addition, *F. alocis* HOT-539_AA69 and *Desulfobulbus* sp. HOT-041_K70 were always detected simultaneously, indicating that there might be a symbiotic relationship between the two taxa. There was no other study reporting the relationship between these two species. Therefore, further studies are needed to confirm the findings.

P. gingivalis (*Pg*) and *T. forsythia* (*Tf*), two species of red complex (Socransky, Haffajee, Cugini, Smith, & Kent, 1998), were also dominating bacteria of GAgP group in this study, which was in consistent with previous research results (Aimetti, Romano, Guzzi, & Carnevale, 2012; Liu et al., 2013). In addition, *Parvimonas micra*, *Peptostreptococcaceae* spp., *Porphyromonas endodontalis*, *Eubacterium nodatum* and *Eubacterium saphenum* were detected more frequently and were more abundant in GAgP group. Previous studies have also reported these bacteria associated with periodontitis (Hashimura, Sato, & Hoshino, 2001; Kumar et al., 2003; Lopez, Dahlen, Retamales, & Baelum, 2011; Mayanagi, Sato, Shimauchi, & Takahashi, 2004; Paster et al., 2001). This study provided further evidence of the role of these species in Chinese patients with GAgP. Although *A. actinomycetemcomitans* has been considered as an important pathogen of AgP (Armitage, 1999, 2004), we did not find it frequently presenting in those GAgP patients. This result was consistent with the study of Li et al. in 2015, which also did not detect *A. actinomycetemcomitans* in Chinese aggressive periodontitis patients (Li et al., 2015).

Several taxa of *Streptococcus*, such as *Streptococcus australis*, *Streptococcus oralis* and *S. cristatus* were present with higher frequency or abundance in healthy control group. They were also reported as health-associated species in other studies (Colombo et al., 2012; Tanner, Maiden, Macuch, Murray, & Kent, 1998), thus indicating those bacteria could be possible protective species in maintaining periodontal health. It was interesting that *P. aeruginosa* was also strongly associated with healthy controls in this study. However, this finding was not consistent with previous studies, which reported *P. aeruginosa* was detected more frequently in chronic periodontitis group (Colombo et al., 2013; Souto, Silva-Boghossian, & Colombo, 2014). Although *P. aeruginosa* strains were considered as transient members of the oral microbiota, they have been reported being detected in significantly higher frequencies in subjects with larger proportions of visible supragingival biofilm, BOP positive sites, PD > 3 mm and clinical attachment loss sites, indicating the relationship of *P. aeruginosa* with inflammation and tissue destruction (Abe, Ishihara, & Okuda, 2001; Colombo et al., 2009; da Silva-Boghossian, do Souto, Luiz, & Colombo, 2011; Paju & Scannapieco, 2007; Souto et al., 2014). The disagreement found in the present study might be due to different populations and sample sizes. Researches with larger sample size and different population will be needed to reach further conclusions.

5. Conclusion

There are more taxa of bacteria in subgingival plaques of Chinese patients with GAgP than healthy controls. *F. alocis*, *Desulfobulbus* sp. and *Fretibacterium* sp. are first time detected as dominant subgingival bacteria in Chinese patients with GAgP. *P. gingivalis* and *T. forsythia* are also strongly associated with GAgP. *Streptococcus* spp. and *P. aeruginosa* may be considered as health-associated species in Chinese population. High-throughout microbiological results may help dentists have a better understanding of subgingival microbiome of GAgP.

Author contributions

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All authors have read and approved the final article.

Conflict of interests

The authors declare that they have no conflict of interests to state.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.archoralbio.2019.02.012.

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