Patterns of subgingival microbiota in different periodontal phenotypes

Short title: Subgingival microbiota in periodontitis patients

ABSTRACT

Objectives: To compare the subgingival microbiota of patients with aggressive (AgP) or chronic periodontitis (CP) to healthy (H), non-periodontitis patients as well as to explore their relevant associations to different host genetic variants.

Methods: Following clinical examination, blood and subgingival plaque sampling of 471 study participants (125 AgP, 121 CP, 225 H), subgingival community analysis was performed by next generation sequencing of the 16S rRNA. Microbial data from 266 participants (75 AgP, 95 CP, 98 H) were available for analysis. SNPs in the *IL6*, *IL6R* and *FTO* gene were selected for genetic marker analyses.

Results: Combined periodontitis patients (AgP + CP), particularly those classified with AgP, exhibited lower alpha- and beta- diversity. Several genera (including *Peptostreptococcaceae, Filifactor, Desulfobulbus, Tannerella* and *Lachnospiracee*) and species were over-abundant in combined periodontitis vs. healthy individuals, while other genera such as *Prevotella* or *Dialister* were found to be more abundant in healthy cases. The only genus with difference abundance between AgP and CP was *Granulicatella*. No associations between *IL6*, *IL6RA* and *FTO* genetic variants and microbial findings were detected.

Conclusion: This study suggests that limited microbial differences existed between AgP and CP and challenges the current notion that periodontitis is associated with increased subgingival microbial diversity compared with periodontal health.

Clinical significance: The findings of this study cast some doubts on the notion that the dysbiosis characteristic of periodontal disease is expressed as increased microbial diversity.

INTRODUCTION

Although the microbial aetiology of periodontal disease was clearly established several decades ago [1], recent advances in scientific methods are leading to a more comprehensive understanding of the role of the microbiota in disease initiation and progression. According to the 'polymicrobial synergy and dysbiosis hypothesis' [2], different members of the microbiota modulate the host response tipping the balance from homeostasis to dysbiosis. It appears that the inflamed environment of the periodontal pocket may favour the growth of inflammophilic bacteria, which in turn can initiate a perpetuating cycle of periodontal disease progression, where dysbiosis and inflammation sustain each other [3]. More recently, a central role for inflammation in determining dysbiosis has been suggested [4]. In this context, host genetic variants affecting the inflammatory response are likely to influence the microbial-inflammation axis, predisposing to microbial shifts leading to periodontitis [5]. Among putative genetic variants suspected to have a role in predisposing to chronic diseases and to microbial dysbiosis, single-genotype polymorphisms (SNP) in genes affecting the inflammatory response (such as in Interleukin genes, or FTO gene variant) and affecting metabolism have been studied [6,7].

Different types of periodontitis, according to the 1999 classification [8], were assumed to be associated with some differences in microbial profiles, with an increase in periodontal pathogens such as *Aggregatibacter actinomycetemcomitans* and *Porhyromonas gingivalis* species in certain populations with aggressive periodontitis (AgP) compared with chronic periodontitis (CP). The role of genetic variants in influencing subgingival microbial colonisation could also vary according to disease sub-types [9].

The aims of this study were to explore the subgingival microbiota of patients with different periodontal phenotypes and to compare the associations between genetic susceptibility markers and detection of subgingival microbes in patients with AgP, CP and healthy periodontia.

MATERIALS AND METHODS

Patient population

In this case-control study, participants were recruited from patients referred to the Eastman Dental Hospital by general dental practitioners. All participants gave written informed consent and the study was reviewed and approved by the Joint UCL/UCLH Committees on the Ethics of Human Research (reference 05/Q0502/84). Participants were recruited between 2005 and 2009.

As previously reported [10], inclusion criteria for periodontitis patients were: i) at least one site with ≥ 5mm probing pocket depth (PPD) and clinical attachment loss (CAL) (except on third molars or distal surfaces of second molars) and ii) no history of periodontal treatment. Inclusion criteria for control subjects (Healthy – H) were: i) absence of any site with ≥ 5mm PPD and CAL (excluding third molars or distal surfaces of second molars) and ii) history of periodontitis and periodontal treatment.

Exclusion criteria for all study participants included (i) known systemic diseases (cardiovascular, respiratory, renal, malignancy, etc.), (ii) history and/or presence of any other infections, (iii) systemic antibiotic treatment in the preceding 3 months, (iv) long-term treatment with any medication suspected to affect the periodontium (e.g. non-steroidal anti-inflammatory drugs), (v) pregnant or lactating females and (vi) <20 teeth present.

Patient characteristics

Self-reported demographic data included age, gender, ethnicity, smoking status and medical history. Patients' height and weight were measured and body mass index (BMI) calculated. Socio-economic factors were obtained from the patients' postcode as quintiles [10] from the Index of Multiple Deprivation 2015, which is the official measure of relative deprivation for small areas in England.

Clinical examination

One calibrated clinician (LN) assessed all subjects and assigned a diagnosis. A comprehensive clinical examination was performed by three examiners, previously calibrated to ≥ 98% agreement for probing pocket depth (PPD) and clinical attachment level (CAL) within 2 mm in repeated measurements of 10 patients each. Full-mouth measures of PPD, gingival recession (REC) and CAL were obtained at six points per tooth. Diagnosis of alveolar bone loss was confirmed by radiographic assessment from each patient. Patients were diagnosed as having localised aggressive periodontitis (LAgP), generalised aggressive periodontitis (GAgP) or chronic periodontitis (CP), according to the 1999 Consensus Classification [8]. The differential diagnosis between AgP and CP was conducted as described before [11]. Briefly, diagnosis of AgP was generally given to systemically healthy patients ≤ 45 years old who had at least 3 teeth with CAL ≥ 6 mm and BOP. Therefore, 'rapid' progression was based on young age, in the absence of previous radiographic records. The cases were further subdivided according to CAL and PPD as no periodontitis, mild, moderate or severe periodontitis according to the American Academy of Periodontology (AAP) 2007 definition [12].

Blood sampling and host DNA analysis

Blood samples were collected from the antecubital vein of the patient sitting on the dental chair in a semi-reclined position. After applying a tourniquet on the biceps area and disinfecting with an alcohol swab, a vacutainer tube system with a butterfly needle was used. Blood samples were stored in a -80°C freezer. The blood sample was then used for DNA extraction and genetic studies of the putative predisposing genes, by allelic discrimination assays using the Applied Biosystems 7300/7500 Real Time PCR System. Single nucleotide polymorphisms (SNPs) in the *Interleukin (IL)6* (rs1800796), *IL6 Receptor (IL6R)* (rs 7529229) and *FTO* gene (rs 1421085) were selected.

Subgingival plaque sampling

Samples of subgingival plaque were taken with sterile curettes inserted to the bottom of the pocket, after careful removal of supragingival plaque and isolation from saliva (gentle air spray or cotton rolls). The 4 deepest sites (one per quadrant) were chosen. After a single stroke each microbiological sample was extracted from the pocket and then immediately pooled and placed into 1 ml of reduced transport fluid and stored at -80°C.

Microbial community and statistical analyses

Subgingival community analysis was performed by next generation sequencing of the 16S rRNA. Briefly, microbial DNA was extracted from subgingival plaque samples and then independent PCR reactions were performed using 785F GGATTAGATACCCBRGTAGTC-3' and 1175R 5'-ACGTCRTCCCCDCCTTCCTC-3' primers fused with Illumina Nextera XT barcode sequences to amplify the V5-V7 hypervariable region on the 16S rRNA gene. The PCR mixtures and conditions has been previously reported [13]. Following amplification reactions, the products were purified (High Stringency Agencourt AMPure Coulter UK) and quantified (Qubit HS DNA kit, Life Technologies, UK) before being combined in equimolar ratios to create a sequencing library. Each of the 7 libraries were sequenced using 250 x 2 paired-end sequencing on a MiSeq sequencer (Reagent Kit v2; Illumina Inc., San Diego, CA, US)[14].

Sequencing data was processed using QIIME 2 (Version 2020.8, https://qiime2.org/) [15]. A total of 426 samples were sequenced across 7 sequencing runs. Raw sequences from each run

were demultiplexed with q2-demux then denoised using DADA2 plugin with default parameters to create Amplicon Sequence Variants (ASVs) [16,17]. After the denoising step, 73 samples with less than 4000 reads and 85 samples originating from one particular sequencing run were excluded. Due to resource and time limitations, it was not possible to re-run these samples. For the downstream analyses all samples were rarefied at 4000 reads. Alpha diversity metrics were calculated using native q2-diversity plugin, and included Number of ASVs, Shannon Index and Faith's Phylogenetic Diversity. For beta diversity, the following two distance matrices were calculated; Aitchison (compositional) and unweighted UniFrac (presence/absence, accounts for phylogenetic information) distances [18,19]. These distance matrices were further analysed using Principal coordinate analysis (PCoA) to investigate clustering patterns based on the metadata groupings. A taxonomic classifier optimised for V5-V7 region of the 16S rRNA gene from the extended human oral microbiome database (HOMD extended, https://tinyurl.com/HOMD-Extended) was trained using q2-feature-classifier, and taxonomy was assigned using this classifier to ASVs at >85% confidence [20]. To determine taxonomic associations between sample groupings at genus and species level, the ALDEx2 package was used in R version 3.6.3. Briefly, this package provides an appropriate framework for differential abundance analysis of compositional data by using the centred log-ratio (CLR) transformation. The ASVs found in <5 samples and had <100 total reads were filtered out from the data set before running the ALDEx2 test. Additionally, correlations between the significantly differentiating taxa and PPD was assessed by calculating Spearman correlation coefficients using Hmisc v.4.3.1 package in R.

The statistical significance of the alpha diversity metrics between healthy and periodontitis (aggressive – AgP, and chronic – CP) groups were tested using paired Kruskal-Wallis test, and $p \leq 0.05$ was accepted as significant. For the 3 SNPs, ANOVA test was used to determine statistical significance (alpha diversity metric ~ disease status + SNP). The clustering of these groups in beta diversity analyses was tested using group-wise PERMANOVA (999 permutations) and $p < 0.05$ was accepted as significant. In addition, adonis test (a multivariate test) was used to investigate variance in relation to metadata groupings as explanatory factors. Finally, for differential abundance testing, Wilcoxon signed-rank test combined with Benjamini-Hochberg correction was used within the ALDEx2 package, and a threshold of $p < 0.01$ was chosen for reporting significantly associated genera and species with healthy or disease [21,22].

The data resulting from the analyses mentioned above was exported from QIIME 2 environment, and respective plots were generated in R using *tidyverse* package v.1.3.0 [23,24]. Descriptive statistics were performed and results were expressed in terms of relative abundance (i.e. percentages) per sample and per group. Stacked bar charts are shown to depict the differences in genus and phylum level distribution between sample groupings.

The sample size calculation was originally based on supposed prevalence of target bacterium *Aggregatibacter actinomycetemcomitans* of 38.5%, 50% and 70% in rare-allele homozygous, heterozygous and common-allele homozygous patients respectively [7], resulting in a total of 226 periodontitis patients needed with α value set at 0.05 and 90% power. The aim was to recruit at least this number of periodontitis patients and an equal number of controls. Data from all participants who took part in the study were entered into a spreadsheet by independent staff not involved in the study, proofed for entry errors and analysed by statistical package IBM SPSS 25.0. Continuous variables are reported as means and standard deviations. The data was summarised, electronically stored and organised in a database (SPSS 22.0 write version and Microsoft Excel Version 14.1.0).

RESULTS

A total of 471 patients took part in the study. Supplemental material 1 shows demographic and periodontal characteristics of the patients by periodontal diagnosis (AgP vs. CP vs. H), as well as distribution of the genotypes for *IL6*, *IL6R* and *FTO* SNPs. Age, gender, ethnicity, smoking and BMI distributions were skewed across the 3 study groups. The AgP group also had more severe disease than CP as measured by number of PPDs > 4 mm (average 64.57 vs 40.44). Genotype distributions satisfied the Hardy-Weinberg equilibrium. Differences in genotype distributions were observed for *IL6R* genotype between AgP, CP and healthy. However, this was likely to be due to ethnic differences among study groups, as no such association was observed in separate ethnic groups (data not reported).

Out of the initial 471 patients, microbial samples were available for analysis for 426. From the 426 sequenced samples, a total of 158 samples were discarded during quality filtering (see supplemental material 2). Out of these samples, 73 were removed due to low sequencing depth after pre-processing, while the remaining 85 were removed due to a distinct taxonomic profile, strongly suggestive of sequencer malfunction. The final sample included 268 patients,

subdivided into 84 AgP, 87 CP and 97 periodontally-healthy subjects (see table 1). This represented 56% of the original sample. No statistically significant differences in studied covariates (age, gender, smoking, ethnicity, BMI, number of PPDs > 4 mm) were observed between the original sample and the sample with valid microbial data. Therefore, the sample with valid microbial data could be considered representative of the overall analysed study sample.

Alpha-diversity

Figure 1 shows microbial diversity data. Decreased diversity was detected in patients with periodontitis (AgP and CP) compared with periodontally-healthy individuals (figure 1a). The Shannon index (figure 1b) showed statistically significant differences ($p<0.001$) for periodontitis (based on AAP criteria) vs. healthy [12]; in the subgroup analysis, diversity was lower for AgP patients compared with both CP ($p=0.008$ Shannon Index) and healthy ($p<0.001$), while the difference between CP and healthy was border-line significant ($p=0.051$). The Faith PD index (figure 1c) revealed no differences for periodontitis vs. healthy ($p=0.56$ for periodontitis vs. healthy), while differences were detected for three-way comparisons between AgP-CP and healthy ($p=0.004$). Observed richness was different for periodontitis vs. healthy ($p=0.003$), with significant differences for AgP-CP-healthy ($p<0.001$). No statistically significant differences in alpha-diversity were detected for gender, ethnicity, smoking, number of PPDs > 4mm and the studied SNPs.

Beta-diversity

Microbial community level differences among the patient groups were revealed by the beta-diversity analyses. The Aitchison distances showed significant compositional shifts between periodontitis vs. healthy ($p=0.001$), and for subgroup analyses AgP-CP-H ($p=0.001$ unadjusted and adjusted) (see figure 1d, 1e). Further analysis using adonis test showed that age and gender were also associated with beta-diversity ($p=0.003$ and $p=0.015$ respectively in the adjusted analysis, figure 1f). Qualitatively, the unweighted-UniFrac distances showed differences between periodontitis and healthy ($p=0.001$ adjusted for confounders) and for disease subgroup analysis ($p=0.001$ adjusted for confounders). Among analysed covariates, only smoking showed significant associations by the adonis test ($p=0.032$). In terms of the 3 studied SNPs, pairwise genotype analyses within each of the periodontitis and healthy groups showed no significant associations at community level for any of the SNP genotypes).

Genera

A total of 17 genera were differently prevalent in periodontitis (defined based on AAP criteria) vs. healthy (at $p < 0.01$) (Figure 2). Relative abundance of 8 genera was higher in periodontitis cases, including *Peptostreptococcaceae XI G-6, G-4, G-5 and G-2, Filifactor, Desulfobulbus, Tannerella* and *Lachnospiracee*, while relative abundance of the following 9 genera was lower in periodontitis cases: *Prevotella, Dialister, Cardiobacterium, Oribacterium, Atopobium, Leptotrichia, Lautropia, Capnocytophaga* and *Campylobacter*. Differences for subgroups AgP-CP-H are presented in supplemental material 3. Only *Granulicatella* genus was significantly different between AgP vs CP groups.

Species

A total of 14 species were differently prevalent in periodontitis vs. healthy (at $p < 0.01$) (Figure 3). Relative abundance of 8 taxa was higher in periodontitis vs. healthy participants: *Tannerella forsythia, Lachnospiraceae G-8 HMT 500, Filifactor alocis, Mogibacterium timidum, Peptostreptococcaceae XI G-2 HMT 369, XI G-5 saphenum, XI G-6 nodatum* and *Desulfobulbus HMT 041*. Six taxa (*Actinomyces sp. HMT 171, Prevotella oris, Oribacterium HMT_078, Dialister invisus, Campylobacter gracilis, Cardiobacterium hominis*) were more abundant in healthy participants compared with periodontitis patients (AgP and CP combined). Periodontopathogenic bacterium *P. gingivalis* was increased in periodontitis vs. healthy, but above the $p=0.01$ threshold ($p=0.035$). No significantly different species level was detected between AgP and CP (data not shown).

Genetic variants-microbial findings

Relative abundance of genera for patients divided by *IL6*, *IL6R* and *FTO* genotypes is reported in Figure 4. No statistically significant associations were detected by genotypes.

DISCUSSION

This study including microbial analysis of 268 participants is one of a limited number of studies with high-throughput techniques assessing the subgingival microbiota. It is also the first, to our knowledge, to assess infectogenomics effects on the subgingival microbiota with this technique in a large patient sample. The main finding is that individuals with periodontitis (AgP and CP

combined) had decreased microbial diversity compared with periodontally-healthy patients. Furthermore, some genera and species were associated with the periodontitis trait.

While increased microbial diversity represents a healthy, stable ecosystem in the gut microbiota [25] and is considered consistent with oral health [26,27], several studies identified increased microbial diversity in periodontitis vs. periodontally-healthy cases [28,29]. This is thought to reflect the complex microbial environment present in biofilms in deep periodontal pockets. In the current study, decreased microbial diversity was detected in periodontitis cases (grouped together based on the 2007 AAP classification) compared with healthy individuals, while no differences were detected for phylogenetic diversity (measured by Faith's index). AgP cases seemed to account for the main difference, exhibiting reduced alpha-diversity and richness. Similarly, beta-diversity, measured by both Aitchison and unweighted-UniFrac analyses, was reduced in periodontitis vs. healthy. Similar findings of a tendency to reduced richness and diversity in both diabetes and periodontitis patients was reported by Farina et al. [30] by whole metagenomic shotgun sequencing of diabetic and non-diabetic patients. Reduced alpha-diversity was also detected in periodontitis (defined as at least 8 teeth with PPD > 5mm and CAL >3mm) compared with periodontal healthy individuals in a metagenomic study including 43 patients [31]. A recent study in Brazilian patients with molar-incisor pattern periodontitis (previously known as localised AgP) showed microbial dysbiosis in periodontal sites, as well as in supragingival and subgingival healthy sites and in stool samples [32]. Another study showed significant differences in alpha diversity between 13 AgP and 13 periodontally healthy patients following 16s rRNA analysis of the subgingival microbiome [33]. Therefore, the issue of association between alpha-diversity and periodontitis still needs to be clarified, and may vary according to populations studied, sample size and methods of analysis employed. When the notion of functional diversity of the microbiota is considered [34], even less is known and needs to be clarified by further studies.

Increased levels of known periodontopathogenic bacteria were found in periodontitis cases compared with healthy individuals in this study. This included genera *Peptostreptococcaceae XI G-6, G-4, G-5 and G-2, Filifactor, Desulfobulbus, Tannerella* and *Lachnospiracee* and species *Tannerella forsythia, Lachnospiraceae G-8 HMT 500, Filifactor alocis, Mogibacterium timidum, Peptostreptococcaceae XI G-2 HMT 369, XI G-5 saphenum, XI G-6 nodatum* and *Desulfobulbus HMT 041*. *P. gingivalis* showed a nominal association with periodontitis. These findings supports knowledge derived by published data, obtained using

low-throughput techniques [35] and highly paralleled pyrosequencing [36,37], which suggested an over-representation of species such as *P. gingivalis*, *T. forsythia* and *T. denticola* in periodontitis cases. Next to this 'traditional' bacteria associated with periodontitis, *Desulfobulbus* and *F. alocis* have clearly emerged as periodontopathogenic bacteria in recent years. *Desulfobulbus* has been previously identified in periodontitis [38] and related to a poor response to periodontal therapy [39]. Aruni et al. [40] showed that *F. alocis* has virulence properties that may favor its ability to survive in the periodontal pocket and that co-culture with *P. gingivalis* may enhance its pathogenicity. Furthermore, *Filifactor* as well as *Mogibacterium* genera were associated with 'persistent' aggressive periodontitis in a recent study [41] and with presence of periodontitis compared with periodontal health in another study [42]. Similar to what is hereby reported, several members of the *Peptostreptococcaceae* genera were increased in buccal and subgingival plaque samples in periodontitis subjects (ChP and AgP) compared with healthy individuals in a recent study in Chinese individuals [43]. With striking similarities with the findings reported here, *Filifactor, Tannerella, Peptostreptococcaceae, Desulfobulbus, Lachnospiraceae, Mogibacterium* were amongst genera increased in the subgingival plaque of 48 periodontitis patients compared with 21 healthy subjects analysed by 16S RNA analysis [44]. In line with the present findings, some members of the *Lachnospiraceae* genera have also been suggested to be associated with periodontitis and gingival inflammation [37,45,46].

Genera *Prevotella, Dialister, Cardiobacterium, Oribacterium, Atopobium, Leptotrichia, Lautropia, Capnocytophaga* and *Campylobacter* and species *Actinomyces sp. HMT 171, Prevotella oris, Oribacterium HMT078, Dialister invisus, Campylobacter gracilis, Cardiobacterium hominis* were more abundant in periodontally-healthy individuals. *Lautropia* had previously reported as health-associated [47,48] and also as associated with 'treated' (vs. 'persistent') AgP [41]. The findings about *Leptotrichia* and *Capnocytophaga* are in contrast with the study above, where they were found in higher abundance in patients with persisting disease [41]. However, *Leptotrichia* has also been consistently associated with periodontal health [42,44,49].

Interestingly, although AgP patients exhibited decreased diversity compared with CP, only minor differences were detected for genera and species in comparison with CP. Only *Granulicatella* genus was significantly different between AgP vs CP groups. This adds to a body of evidence comparing microbial patterns in these two forms of periodontitis [43,50–52]. Previous systematic reviews have suggested that, despite an association of *A.*

actinomycetemcomitans with AgP, no species could differentiate between CP and AgP [53,54]. These findings lend support to the elimination of these terms (AgP and CP) from the current classification, based on clear absence of evidence of different pathogenic pathways [9].

Analysis of association between genetic variants and subgingival microbes was limited here to three SNPs, located in the *IL6* gene, the *IL6R* gene and the *FTO* gene. The rationale for the choice of these genetic variants was based on preliminary evidence of association between *IL6* SNPs and periodontopathogenic bacteria [7] and the aim to expand to study any potential effect of the *IL6R* gene. The addition of the *FTO* gene was based on its potential effect on metabolic disorders and periodontal status [6]. Other genetic variants have since emerged as potentially associated with colonisation by specific oral bacteria [7,55]. No obvious associations between the studied genetic variants and the composition of the subgingival microbiota were detected in this study, except a border-line difference in beta-diversity between *IL6* CC and GG genotypes. This first large study on infectogenomics employing 16s analysis of the subgingival microbiota confirms the difficulty to obtain a clear picture of genetic variants associated with subgingival microbial composition. In this context, it is important to stress that, although the number of patients included in the study (n=471) slightly exceeded the sample size calculation (n=226 periodontitis and an equal number of controls), due to a large number of discarded samples, the study may be under-powered to test the genetic variant-microbes associations. While high-throughput -omics studies to study both host genetic variants and the subgingival biofilm are advisable, challenges and limitations in processing, analysing and interpreting such complex data should be recognised. Another possible area of future research would be to investigate associations between functional genes and the oral microbiome with high-throughput sequencing.

Among other factors analysed, only smoking showed a weak association with beta diversity, although the study was not powered to test these factors. Smoking is thought to influence the composition of the subgingival microbiota and to be associated with lower microbial diversity [42]. In particular, smoking may be associated with specific pathogen-rich communities in the subgingival and peri-implant biofilms [56,57].

This study suffers from a number of important limitations. First of all, many microbial data were lost due to technical challenges with the microbial analysis and only just over half of initial sample analysed yielded valid microbial data. This is mitigated by the fact that patients

with valid microbial data appeared to be representative of the overall sample, since no clear differences in demographic and clinical variables could be detected. Furthermore, this study protocol was written in 2005 and recruitment completed in 2009. Therefore, the 1999 classification was used and not all data are now available for retrospective assignment of the new classification. However, the 2007 AAP classification to distinguish periodontitis cases was also used, according to the study protocol, and it is used to report microbial associations, as previously done in other similar studies [58]. The use of the new classification [9] would likely not change the results relative to combined periodontitis vs. healthy participants but would affect the observed chronic vs. aggressive periodontitis findings. The strengths of this study lie in the much larger sample size than most other published studies in this field (albeit reduced due to the issues discussed above), and in the addition of the investigation of host genetic variants.

Overall, this study brings forward confirmatory as well as novel evidence on the association between certain genera and species with presence of periodontitis, suggesting that limited microbial differences existed between the previous categories of AgP and CP and casts some doubts over the current notion that periodontitis is associated with increased subgingival microbial diversity compared with health. Further metagenomic studies, possibly associated with metabolomics and host genetic analyses, can provide more knowledge in the fast-evolving topic of periodontal host-microbial interactions.

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		AgP (*n*=75)	CP (*n*=95)	Healthy (*n*=98)	P value for comparisons across groups
Gender	Male	23 (30.7%)	35 (36.8%)	46 (46.9%)	0.072
	Female	52 (69.3%)	60 (63.2%)	52 (53.1%)	
Smoking Status	Never	40 (53.3%)	48(50.5%)	72 (73.5%)	0.009
	Current	23 (30.7%)	26 (27.4%)	15 (15.3%)	
	Former	12 (16.0%)	21 (22.1%)	11 (11.2%)	
Ethnicity	Caucasian	32 (42.7%)	57 (60.6%)	67 (69.1%)	<0.001
	Asian	8 (10.7%)	18 (19.1%)	19 (19.6%)	
	African	14 (18.7%)	1 (1.1%)	6 (6.2%)	
	Afro-Caribbean	17 (22.7%)	15 (16.0%)	2 (2.1%)	
	Other	4 (5.3%)	5 (3.21%)	3 (3.1%)	
Age		34.05 ±6.39	45.76 ±9.21	37.34 ±11.27	<0.001
BMI		26.94 ±6.80	26.49 ±5.00	24.26 ±4.17	<0.001
No. PPD >4 mm		64.57 ±39.82	40.44 ±25.34	0.34 ±0.97	<0.001
IL6 rs1800796	CC	8 (10.8%)	10 (11.0%)	13 (13.4%)	0.279
	CG	16 (21.6%)	33 (36.3%)	31 (32.0%)	
	GG	50 (67.6%)	48 (52.7%)	53 (54.6%)	
IL6R rs7529229	CC	22 (31.4%)	12 (12.9%)	19 (19.6%)	0.053
	CT	30 (42.9%)	47 (50.5%)	42 (43.3%)	
	TT	18 (25.7%)	34 (36.6%)	36 (37.1%)	
FTO rs1421085	CC	7 (9.7%)	11 (12.6%)	10 (10.9%)	0.402

CT	24 (33.3%)	31 (35.6%)	43 (46.7%)
TT	41 (56.9%)	45 (51.7%)	39 (42.4%)

Table 1. Characteristics of included cases with valid microbial data, divided by periodontal diagnosis. For categorical data total numbers and frequency within classification is shown. For continuous data mean values and standard deviation are shown. Comparisons between categorical and continuous data were analysed with Chi squared and ANOVA, respectively. ID= Index of Multiple Deprivations; BMI= body mass index; FMPS= Full Mouth Plaque Score; FMBS= Full Mouth Bleeding Score; PPD= Probing Pocket Depth.

FIGURE LEGENDS

Figure 1. Microbial diversity in health and periodontitis. Alpha diversity metrics of richness (a), evenness (b) and phylogenetic diversity (c) showed significant differences between groups. Community level diversity analysis by PCoA using both unweighted UniFrac (d) and Aitchison (e) distance matrices showed significant community shifts. Adonis test (f) further revealed that the periodontitis diagnosis was the best explanatory factor for the observed variability in community diversity as shown by the PCoA plots. The following groupwise comparisons were performed for alpha (Kruskal-Wallis test) and beta (PERMANOVA) diversity metrics; a, healthy vs periodontitis; b, healthy vs aggressive periodontitis (AgP); c, healthy vs chronic periodontitis (CP). *, $p<0.05$; **, $p\leq0.01$; ***, $p\leq0.001$.

Figure 2. Genera Genus level significant associations across healthy and periodontitis groups. The Aldex2 test results revealed that 9 genera had an increased abundance in patients with periodontitis while the relative abundance of 8 genera was higher in healthy patients. CLR transformed relative abundances (a) and median differences (b) along with correlation to the PPD (c) for each genus is shown for each of the significantly different genus.

Figure 3. Species level significant associations across healthy and periodontitis groups. The Aldex2 test results revealed that 8 genera had an increased abundance in patients with periodontitis while the relative abundance of 6 genera was higher in healthy patients. CLR transformed relative abundances (a) and median differences (b) along with correlation to the PPD (c) for each genus is shown for each of the significantly different genus.

Figure 4. Genus level taxonomic profiles of the SNP genotypes showing the top 20 genera across all patients.