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Research Report

Oral Dysbiosis and Inflammation in Parkinson's Disease

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Abstract.

Background: Oral microbiota has largely escaped attention in Parkinson's disease (PD), despite its pivotal role in maintaining oral and systemic health.

Objective: The aim of our study was to examine the composition of the oral microbiota and the degree of oral inflammation in PD.

Methods: Twenty PD patients were compared to 20 healthy controls. Neurological, periodontal and dental examinations were performed as well as dental scaling and gingival crevicular fluid sampling for cytokines measurement (interleukine (IL)-1β, IL-6, IL-1 receptor antagonist (RA), interferon-γ and tumor necrosis factor (TNF)-α). Two months later, oral microbiota was sampled from saliva and subgingival dental plaque. A 16S rRNA gene amplicon sequencing was used to assess bacterial communities.

Results: PD patients were in the early and mid-stage phases of their disease (Hoehn & Yahr 2–2.5). Dental and periodontal parameters did not differ between groups. The levels of IL-1β and IL-1RA were significantly increased in patients compared to controls with a trend for an increased level of TNF-α in patients. Both saliva and subgingival dental plaque microbiota differed between patients and controls. *Streptococcus mutans*, *Kingella oralis*, *Actinomyces* AFQC_s, Veillonella AFUJ_s, *Scardovia*, Lactobacillaceae, Negativicutes and Firmicutes were more abundant in patients, whereas *Treponema* KE332528_s, Lachnospiraceae AM420052_s, and phylum SR1 were less abundant.

Conclusion: Our findings show that the oral microbiome is altered in early and mid-stage PD. Although PD patients had good dental and periodontal status, local inflammation was already present in the oral cavity. The relationship between oral dysbiosis, inflammation and the pathogenesis of PD requires further study.

Keywords: Oral microbiome, inflammation, biomarker, non-motor symptoms, cytokine, Parkinson's disease, microbiota

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INTRODUCTION

The etiology of Parkinson's disease (PD) is unknown in the vast majority of cases but probably involves a complex interaction between genetic predisposition, environmental contributors and age-related processes [1]. The hypothesis that the periphery of the body could be an early site of PD comes from the observation that phosphorylated (phos-) α-synuclein aggregates [2], the histopathological hallmark of PD, are found not only in the central nervous system (CNS) but also within neurons of the olfactory bulb and in the peripheral autonomic nervous system of the upper aerodigestive [3] and gastrointestinal tracts, very early in the course of the disease, before the classic motor symptoms emerge [4–6]. Consequently, PD patients frequently present a history of hyposmia, oral difficulties and/or gastrointestinal dysfunction [7], sometimes years before being diagnosed with PD.

Little attention has been paid to the role of the mouth in the PD literature. However, a rostro-caudal gradient of decreasing phos-α-synuclein histopathology has been demonstrated, with the highest density being found in the submandibular glands and the lower esophagus, and the lowest density in the colon and the rectum [8]. Hence, submandibular gland needle biopsies have recently been proposed as a method for the diagnosis of PD [9–11]. Oral phos-α-synuclein deposits may underlie non-motor symptoms (NMS) [12] such as decreased salivary production and dysphagia [7]. These symptoms typically become troublesome in more advanced PD, but they can develop early, occasionally as the presenting feature [13, 14]. A higher frequency of poor oral health, caries and periodontal disease, and fewer remaining teeth have been reported in PD [15–17].

Recently, attention has increasingly been focused on the microbiota as a new player in PD pathogenesis because of its key role in the protection of the host from pathogenic organisms [18]. Several studies showed changes in PD patients' gut microbiota with an important variation in the reported taxa [19–24]. Gut dysbiosis promotes intestinal inflammation and increased mucosal permeability [25–29], inducing or maintaining excessive phos-α-synuclein expression and misfolding [25, 30–35]. Phos-α-synuclein aggregates could then migrate, from the peripheral nervous system to the CNS through trans-synaptic transmission and from cell-to-cell in a prion-like fashion [36], eventually leading to PD degeneration.

In contrast to gut microbiota, data on oral microbiota in PD are scarce [37, 38]. However, the study of the oral microbiota may offer several advantages: 1) sampling is easy; 2) it represents the second largest and second most diverse microbiota after the gut, harboring over 700 species of bacteria [39]; 3) it is one of the microbiota which has the least intrapersonal variability, enabling broader conclusions [40]; 4) it is strategically located at the crossroads between the outside environment, the respiratory and digestive tracts. As such, the mouth is one of the first structures to be exposed to the external environment, such as airborne and diet-contained organisms, and is consequently one of the primary sites of entry for pathogenic microorganisms; and 5) oral microbiota is essential to maintain oral and systemic health [41]. Oral dysbiosis is associated with oral diseases such as dental caries and periodontal disease, but also with systemic diseases such as infective endocarditis, atherosclerosis [42] and with multifactorial neurodegenerative conditions such as glaucoma [43].

Considering the early involvement of the upper aerodigestive tract in terms of PD non-motor symptoms and α-synuclein deposits, we speculated that the study of the mouth could be central to improved understanding of PD pathophysiology. We studied the salivary and subgingival dental plaque microbiota, by comparing bacterial diversity and taxonomic composition in PD patients and matched healthy subjects. We also compared the level of oral inflammation between patients and controls, which was assessed both clinically and biologically.

METHODS

The study was approved by the Geneva Ethics Committee (EC-2016-01680). All participants gave written informed consent.

Study population

Patients were recruited from the Movement Disorders Unit of Geneva University Hospital. Selection criteria were a diagnosis of PD [44] made after 50 years of age (Supplementary Material A). Patients were compared with age-, sex-, and body mass index-matched healthy controls. Exclusion criteria included conditions that could independently affect the oral microbiome (Supplementary Material A).

Clinical data

The clinical assessment included the Movement Disorder Society-Unified Parkinson's Disease Rating Scale [45] part III for motor PD symptoms and the Hoehn & Yahr (H&Y) scale [46] for PD staging, the NMS Quest [47] for non-motor PD symptoms, the Hospital Anxiety and Depression scale [48] for the levels of anxiety and depression and the Montreal Cognitive Assessment (MoCA) [49] for the degree of cognitive impairment. The degree of drooling was assessed by the Sialorrhea Clinical Scale for PD [50], the degree of hyposialorrhea by the paraffin-stimulated salivary flow rate [51], the level of dysphagia by the Swallowing Disturbance Questionnaire [52] and the degree of constipation by the Bristol Stool Form Scale [53]. The dental and periodontal screening included an interview to assess oral hygiene, dental history, and socioeconomic background. The clinical oral assessment included counting the number of decayed, missing and filled teeth [54] (which determines the prevalence of dental caries as well as dental treatment needs) and the plaque index [55], which records the presence and quantity of dental biofilm that accumulates on the surface of the tooth. The periodontal inflammatory status was clinically assessed by probing pocket depth and the bleeding on probing [56]. A standardized dental scaling was performed at the end of the session in all participants ("reset" of the oral cavity) in order to control for oral hygiene. Advice about dental hygiene, teeth brushing and avoidance of oral antiseptics was given. All participants received the same toothbrush and toothpaste.

Cytokine dosage

Oral inflammation was also assessed by measuring interleukin (IL)-1β, IL-6, IL-1 receptor antagonist (IL-1RA), interferon (IFN)-γ and tumor necrosis factor (TNF)-α concentrations in the gingival crevicular fluid using Bio-Plex cytokine multiplex assays [57].

Analysis of oral microbiota

Two months after the "reset" of the oral cavity, microbiota sampling was performed from two different oral sites: the saliva and the subgingival dental plaque (i.e., tooth biofilm below the gum). Participants were asked to avoid teeth brushing the morning of the sampling. Unstimulated saliva was obtained by spitting into a sterile plastic 50 mL tube in the morning, at least 1.5 h after eating. Subgingival dental plaque was obtained by a pooled sample from the deepest pocket in each quadrant using sterile paper points. Within the following 3 h, samples were transferred for storage and kept at a temperature of –80°C until further processing. The principal steps in our analysis of bacterial communities in saliva and dental plaque were [58]: 1) DNA extraction; 2) bacterial DNA quantification using quantitative polymerase chain reaction (qPCR); and 3) Illumina sequencing of amplicons generated from the V3–4 region of the bacterial 16S ribosomal RNA genes. Merged sequence reads were clustered into zero-radius operational taxonomic units (zOTUs) with a method [59] resolving differences as small as one nucleotide and providing taxonomic resolution superior to conventional 97% OTUs [60]; 4) comparison of zOTUs to the EzBioCloud [61] 16S rRNA gene database for taxonomic assignments; 5) microbial communities comparisons in regards to ecological indices (richness, diversity) and abundance of taxa from phylum to zOTU level; and 6) assessment of correlations between microbial composition and clinical outcomes (Supplementary Material A).

Statistical analysis

For univariate comparisons, we used the Wilcoxon rank sum test, unless otherwise indicated. For multivariate analyses of the microbiota, we constructed the Bray-Curtis similarity [62] matrix based on square root transformed relative abundance of zOTUs. In principal coordinates analysis (PCoA, PRIMER) each sample was visualized in a multidimensional space as a point whose location reflected its microbial composition (i.e., percentage of different zOTUs). To assess differences in overall microbiota taxonomic composition between groups defined by categorical variables (e.g., PD vs. controls), we used permutational multivariate analysis of variance (PERMANOVA; PRIMER v7, PRIMER-E Ltd, Plymouth, UK) with 9,999 permutations. Canonical analysis of principal coordinates (CAP, PRIMER) was used to test the success of allocating subjects to PD and controls from specific microbial profiles. This method maximizes the group differences in the multivariate cloud of points (constrained ordination) [63], and calculates the proportion of correct classifications of the groups according to the PD or control status. The permutational analysis of multivariate dispersions (PERMDISP, PRIMER) was performed (with 9,999 permutations) to examine homogeneity

of multivariate dispersion (distances to centroids) of microbial communities between patients and controls. To analyze the relationship between bacterial community profiles and quantitative clinical continuous variables, we used a distance-based linear model (DISTLM, PRIMER) with 9,999 permutations.

To assess statistical significance of differences in the relative abundance of individual taxa according to the PD status, we used DESeq2 [64]. The taxa found in less than 25% ($n = 10$) of compared samples were excluded. FDR corrected p values < 0.05 were considered significant. DESeq2 models were additionally run with adjustment for age category and sex. Age was categorized in two groups: above-median and below-median.

RESULTS

Clinical data

A total of 40 participants were recruited, including 20 PD patients and 20 healthy controls. Demographic and clinical characteristics of each group are reported in Table 1 and Supplementary Material B. Participants were similar regarding the level of education, comorbidities, cognitive status and anxiety level. No clinical differences were identified in terms of dental or periodontal status. PD patients were predominantly within the early and mid-stage phases of their disease (median from PD diagnosis = 4.7 years, H&Y range from 2 to 2.5). As expected, PD patients had more severe motor and non-motor PD symptoms compared with controls. PD patients had significantly higher swallowing and saliva disturbances scores than controls, although their scores themselves did not reach pathological cut-offs. All but two PD patients were taking dopaminergic replacement therapy. One patient was treated with deep brain stimulation (Table 1 and Supplementary Material B).

Cytokines in the gingival crevicular fluid

The levels of cytokines IL-1β and IL-1RA were significantly increased in PD patients compared to controls, with a trend for an increased level of TNF-α in PD patients. No difference was found in the concentrations of IL-6 and IFN-γ between the two groups (Table 2).

Microbiome data

The number of reads obtained by sequencing of bacterial 16S rRNA gene amplicons is provided in Supplementary Material C. The relative abundance of 1,613 zOTUs identified was used to calculate similarities between bacterial communities, which we visualized using PCoA. Saliva and plaque samples were clearly separated in the first two PCo axes (Supplementary Material D, Supplementary Figure 1, Supplementary Table 1). The inter-site (i.e., saliva vs. plaque) microbiota Bray-Curtis similarity, in the same individual mouth was somewhat higher in patients than in controls [median (Q1; Q3) 47.9 (45.9; 56.9) vs. 43.7 (40.7; 50.5)], and this difference was close to the threshold of statistical significance ($p = 0.056$). Bacterial load expressed as the number of 16S rRNA copies in DNA extract did not show significant differences between PD and control groups in either saliva [median (Q1; Q3) 2.73 (1.17; 5.5) $\times 10^7$ vs. 4.81 (1.44; 15.72) $\times 10^7$; $p = 0.14$)] or plaque [2.4 (1.4; 4.13) $\times 10^7$ vs. 2.57 (1.84; 3.52) $\times 10^7$; $p = 0.98$)] samples.

We assessed microbiota differences between controls and patients separately for saliva and dental plaque samples. Salivary microbiota profiles were different between patients and controls (PERMANOVA, $p = 0.006$) (Fig. 1A). The two arbitrarily defined non-overlapping clusters respectively contained 75% (15/20) of samples from patients and 80% (16/20) of control samples. Differences in dental plaque microbial communities between PD and control subjects were also present (PERMANOVA $p = 0.03$) with two separate clusters in PCoA, each formed by 75% (15/20) of samples from individuals with the same disease status (Fig. 1B). CAP, which is a constrained ordination method, allowed to allocate samples according to the PD status with 72.5% success for both saliva (correct classification of 75% PD patients and 70% controls) and plaque (correct classification of 65% PD patients and 80% controls) microbiota. Of note, saliva and plaque microbiota from 5 patients (#3, #4, #5, #10, #11) were misallocated to the control group using CAP. The results of the PERMDISP analysis showed that multivariate dispersion of microbial communities of patients and controls were not significantly different. No significant differences of oral microbiota were found between sexes in either sample type.

Compared with controls, PD patients presented with a lower alpha-diversity, as measured by species richness (Fig. 2A) and Shannon diversity index (Fig. 2B) in the dental plaque. The same trend was also observed in saliva samples.

Relative bacterial taxa composition was significantly different between PD patients and controls.

Table 1
Selected demographic and clinical characteristics of participants including all parameters that showed significantly different distributions between groups

	PD Patients	Controls	p
Demographics			
Number of participants [men (%)]	20 [9 (45%)]	20 [9 (45%)]	1 (F)
Age (y)	62.8 (60.1; 67.0)	64.3 (59.2; 68.3)	0.8
Education level I, II, ≥ III	0, 3, 17 (0%, 15%, 85%)	1, 2, 17 (5%,10%,85%)	0.5 (C)
Body Mass Index (kg/m^2)	24.8 (20.8; 28.8)	25.8 (23.8; 26.9)	0.8
Current smokers (%)	0 (0%)	0 (0%)	1 (F)
Former smokers (%)	4 (20%)	5 (25%)	1 (F)
Parkinson's disease duration (y)	4.7 (3; 7.4)		
Hoehn & Yahr stage (/4)	2 (2; 2.5)		
Clinical symptoms			
Motor score MDS-UPDRS III (/132)	15 (11.5; 20.5)	0 (0; 0)	< 0.001
Non-motor score NMSQ (/30)	11 (6.7; 14)	3 (2; 4.2)	< 0.001
Swallowing Disturbance Questionnaire (SDQ) score (/45)	3 (1.5; 5.5)	0.5 (0.5; 0.5)	< 0.001
Sialorrhea Clinical Scale score (/21)	1.5 (0; 3.2)	0 (0; 0)	< 0.001
Paraffin-stimulated salivary flow rate (ml/min)	1.6 (0.8; 1.7)	6 (6; 6)	< 0.001
Constipation level Bristol Stool Form Scale score (/7)	3 (1.7; 3)	4 (3; 4)	< 0.001
Cognitive score MoCA (/30)	29 (28.7; 30)	30 (28; 30)	0.4
Anxiety HAD score (/21)	4 (3; 7.2)	4.5 (2.7; 6.2)	0.5
Depression HAD score (/21)	3 (2.7; 6.2)	2 (1; 4)	0.02
Dental and periodontal parameters			
Participants with tooth brushing > once/day (%)	20 (100%)	20 (100%)	1 (F)
Participants with dental visits ≥ once/year (%)	20 (100%)	20 (100%)	1 (F)
Participants with decayed teeth (%)	2 (10%)	2 (10%)	1 (F)
Percentage of filled teeth	49.05 (33.3; 55.6)	55.5 (34; 64.1)	0.425
PPD (mm)	2.99 (2.9; 3.1)	3 (2.8; 3.1)	0.766
Plaque index	1.29 (0.8; 1.4)	0.97 (0.8; 1.2)	0.218
BOP	57.91 (34.1; 69.9)	51.52 (34.6; 59.7)	0.725
Percentage of PPD ≥ 4 sites	13.12 (7.6; 20.7)	8.04 (5.4; 17.1)	0.465
Percentage of PPD ≥ 4 BOP + sites	11.11 (5.6; 19.5)	6.85 (1.8; 15.1)	0.351
Medication			
Levodopa-equivalent daily dose (mg/d)	522.5 (296.9; 720.6)	0 (0; 0)	< 0.001
Number of patients on Levodopa (%)	16 (80%)	0	< 0.001 (F)
Number of patients on Dopamine agonist	12 (60%)	0	< 0.001 (F)
Number of patients on MAO Inhibitor	6 (30%)	0	0.02 (F)

For values expressed as median (Q1; Q3) scores, Wilcoxon rank sum test was used. When values were expressed as number of participants, % of the participant's group was indicated in brackets and Fisher's exact test (F) or Chi-square test (C) were used. Education level: Level I is defined as subjects who received a primary education, Level II a lower secondary education, and level 3 and above at least an upper secondary education. A total Swallowing Disturbance Questionnaire SDQ score of ≥ 11/45, a paraffin-stimulated salivary flow rate value < 0.7 ml/min and a HAD Depression score ≥ 8/21 were considered to be pathological cut-off values for dysphagia, hyposialorrhea, and depression respectively. BOP, bleeding on probing; BOP +, presence of BOP; HAD, Hospital Anxiety and Depression scale; MDS-UPDRS III score, motor scale of Movement Disorder Society Unified Parkinson's Disease Rating Scale; MoCA, Montreal Cognitive Assessment; NMSQ, Non-Motor Symptoms Quest; PD, Parkinson's disease; PPD, probing pocket depth.

Table 2
Levels of cytokines in the gingival crevicular fluid in PD patients and controls

	PD patients	Healthy controls	p
IL-1β (pg/ml)	61.6 (16.5; 107.4)	22.4 (8.8; 35.4)	0.05
IL-6 (pg/ml)	4.4 (2.3; 10.8)	2.5 (1.6; 4.6)	0.20
IL-1RA (pg/ml)	44,247.5 (28,736.7; 92,068.9)	29,153.0 (20,227.3; 42,257.5)	0.04
IFN-γ (pg/ml)	2.0 (1.3; 2.8)	1.8 (1.4; 2.7)	0.93
TNF-α (pg/ml)	16.5 (9.1; 38.1)	9.1 (5.2; 18.8)	0.06

Values are expressed as median (Q1; Q3) scores.

Supplementary Table 2 summarizes the taxa significantly different in abundance between patients and controls, from phylum to zOTU level. When a significant differential abundance according to the PD status was found in one sample type (i.e., saliva or dental plaque), the same trend (i.e., decrease or
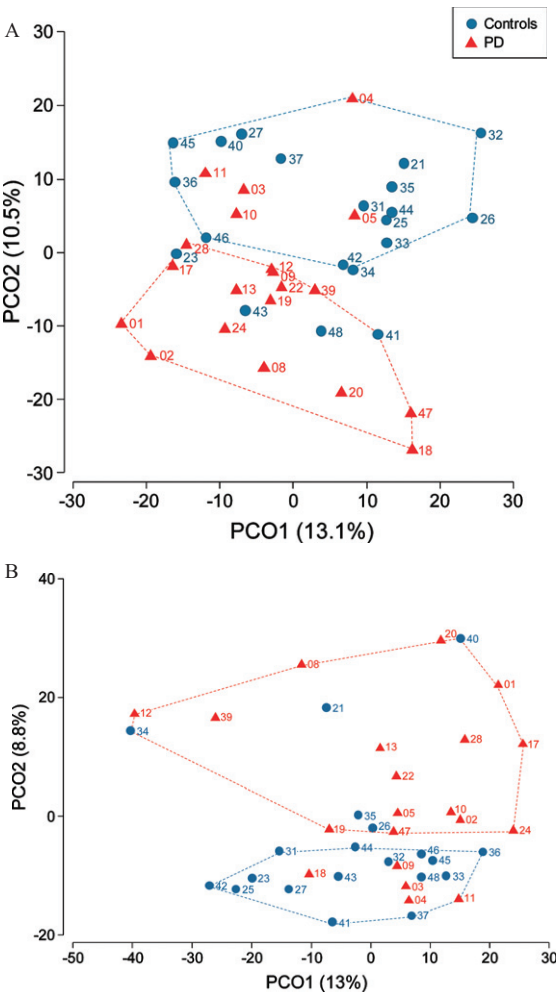
Fig. 1. Differential bacterial community composition between controls and PD patients assessed in saliva (A) and dental plaque samples (B). Participant's number is indicated next to her/his location point.

Fig. 2. zOTU richness (A) and Shannon bacterial diversity index (B) in saliva and dental plaque samples in PD patients and controls.

increase) was commonly (81.7%) observed in the other sample type. This was notably the case for 23 of 25 taxa (92%), whose abundance was significantly different between patients and controls in both saliva and dental plaque (Fig. 3). The phylum Firmicutes, class Negativicutes, family Lactobacillaceae/genus *Lactobacillus* and genus *Scardovia* (Actinobacteria) had higher relative abundance in PD patients. Of seven species or phylotypes (i.e., so far uncultured species) discriminating between PD and controls, four were more abundant in PD patients: *Actinomyces* AFQC_s, *Veillonella* AFUJ_s, *Kingella oralis* and *Streptococcus mutans*. Three zOTU from these species/phylotypes, as well as one from each unclassified *Veillonella* and *Alloprevotella*, also had increased proportion in PD patients. The taxa that had
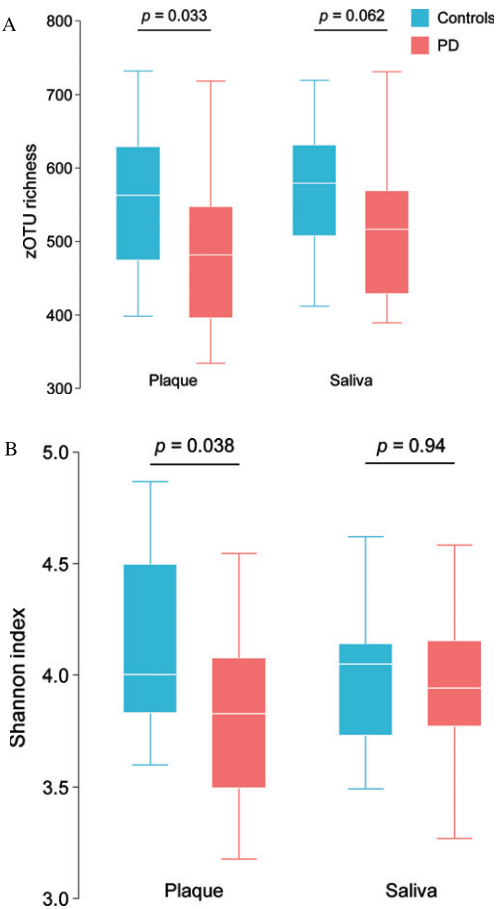
significantly lower relative abundance in PD patients included: zOTU290 (*Leptotrichia),* the phylotypes AM420052_s (Lachnospiraceae), KE332528_s (*Treponema*) and, JX294356_s and the higher-level taxa (up to the phylum SR1) to which the latter phylotype belongs. The observed associations between microbial taxa and PD status remained significant after adjustment for age category and sex (Supplementary Table 2) in the majority of cases.

We calculated the 'absolute abundance' of bacterial taxa, by combining their relative abundance (from the 16S sequencing data) with the number of 16S rRNA gene copies (determined by qPCR). Comparison between PD patients and controls revealed 194 differentially abundant taxa in either or both sample types (Supplementary Table 3). Importantly, of 44 taxa with significant differences (non-corrected *p* values) in both saliva and plaque, 41 had higher abundance in controls, while *S. mutans*, *S. mutans* zOTU309 and *Streptococcus* zOTU112 were more

abundant in PD patients. After correction for multiple testing, the only differences that remained significant were decreased abundances of phyla Tenericutes and SR1 in saliva samples of PD patients.

Correlations between microbiota composition and clinical data

Regarding correlations between whole microbiota composition and clinical data (Supplementary Table 4), DISTLM analysis showed associations between salivary microbiota of PD patients and MoCA score, salivary flow rate, and parameters indicating an increased risk of periodontal disease. A trend for an association was found between PD salivary microbiota and NMS Quest score, anxiety and depression scores. PD dental plaque microbiota correlated with age at PD onset. In saliva samples from PD patients, Spearman correlations showed a positive correlation between Actinomycetales and tremor scores and between *Veillonella* and dysphagia (SDQ) score (Supplementary Table 5).

DISCUSSION

PD patients exhibited a distinct microbiota profile in their saliva and subgingival dental plaque compared to controls. PD patients had a lower alpha-diversity in plaque samples with a similar trend observed in saliva. The level of pro-inflammatory cytokine IL-1β measured in the gingival crevicular fluid was significantly increased with a trend for an increased level of TNF-α in PD patients compared to controls, although PD patients had a good dental and periodontal status. Microbial profiles in saliva (but not in dental plaque) were associated with the cognitive status, salivary flow rate and indexes indicating an increased risk of periodontal disease. Dental plaque microbiota was related to PD motor symptoms severity.

To the best of our knowledge, this is the first time that the subgingival dental plaque has been studied in PD patients. To date, only two studies compared oral microbiota in PD patients and controls [37, 38]. Pereira et al. [37] studied samples originating from buccal and sublingual mucosa whereas Mihaila et al. [38] used salivary samples. We performed a detailed oral and periodontal examination, a good control of oral hygiene by means of the "reset" of the oral cavity, and cytokine measurement in the gingival crevicular fluid which reflects the level of local inflammation, all

of which was not performed in the above mentioned studies. This allowed us to precisely assess the inflammatory state of the oral cavity. In accordance with the two previously mentioned studies, we demonstrated that PD patients had a different oral bacterial ecology than controls. We found similar results regarding increases in the relative abundance of *S. mutans* [38, 65], *Veillonella* [37], Lactobacillaceae [37, 38] and *Scardovia* [38] in PD. In addition, we found that *K. oralis* and Negativicutes (a class withing the phylum Firmicutes, represented in particular by *Veillonella*), as well as the phylum Firmicutes were increased and that the phylum SR1 was decreased in PD patients. *In vitro* experiments have demonstrated that a species from the genus *Veillonella* reduces the growth inhibition of *S. mutans* [66] by antagonistic streptococcal species.

The bacteria found in increased proportions in our PD patients have been implicated in oral pathologies such as dental caries (*S. mutans* [67], *Lactobacillus* [68] and *Scardovia* [69]) and periodontitis (*K. oralis* [70] and Negativicutes [71]). Our patients did not present with more decayed teeth, nor more periodontitis than controls, probably because they were in the early and middle phases of their disease with low motor scores, allowing good fine hand motor skills [72] and no cognitive impairment nor significant depression, anxiety or apathy, all ensuring the capacity to maintain good oral hygiene. However, a higher frequency of caries and periodontal disease has been reported in PD [15–17]. In that light, our results could then suggest that the changes observed in PD oral microbiota composition could correspond to a transition phase in the development of oral diseases. More importantly, we believe that our data support the notion that an altered oral microbiome is unlikely a consequence of dental or periodontal complications, but rather may be a primary event, somehow related to the pathogenesis of PD. This hypothesis, however, requires further study for confirmation.

Interestingly, *S. mutans*, whose relative abundance was increased in our patients, is capable of amyloid formation [73]. Bacterial amyloid production contributes to the biology of numerous micro-organisms, with particular relevance for adhesion and biofilm formation. While the link between microbial amyloid formation and PD remains to be demonstrated [74], it has been shown in animal models that bacterial amyloids may play a role in alpha-synuclein production and aggregation, as well as cerebral inflammation. Rats orally exposed to amyloid-producing bacteria presented with enhanced alpha-synuclein produc-
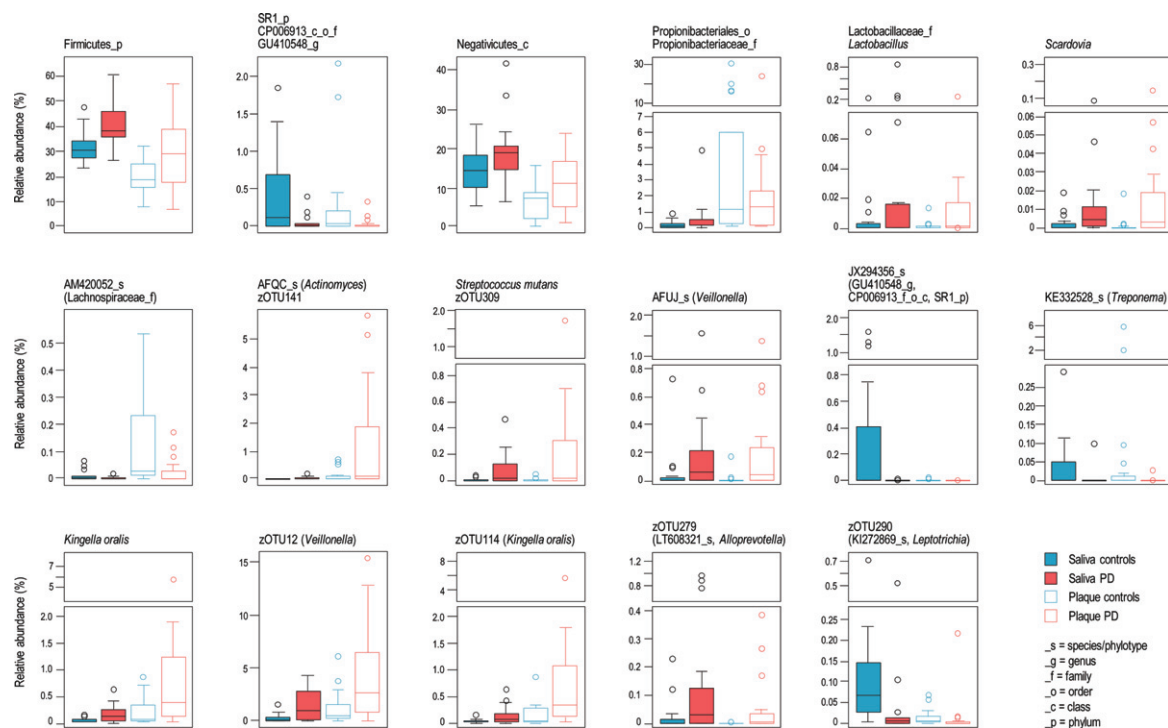
Fig. 3. Bacterial taxa significantly different in abundance between patients and controls in both saliva and dental plaque.

tion in the gut, as well as an increased production and aggregation of alpha-synuclein in the brain. Enhanced cerebral inflammation was also demonstrated in rats orally exposed to amyloid-producing *E. coli* when compared with animals exposed to isogenic *E. coli* mutant lacking the ability to produce bacterial amyloid protein [75]. Using a mice model for PD, Sampson *et al.* showed that gut microbiota was needed for motor impairment, alpha-synuclein pathology and microglia activation [30], and all of these were improved by the use of antibiotics and worsened by microbial re-colonization. The pathway of the innate immune system recognizing bacterial amyloid as a pathogen-associated molecular pattern [76], is also involved in the recognition of misfolded alpha-synuclein [77]. Fecal transplants from PD patients performed in alpha-synuclein-overexpressing mice enhanced physical impairments compared to microbiota transplants from healthy human donors [30]. Recently, a Finnish nationwide case-control study demonstrated that prior exposure to certain antibiotics was associated with an increased risk of PD with a delay consistent with the duration of the prodromal period. Changes in microbiota composition secondary to the exposure to certain antibiotics could explain the increased risk of PD [78].

Pro-inflammatory cytokine levels were significantly increased in PD gingival crevicular fluid. The level of IL-1RA was also increased. The level of this anti-inflammatory cytokine goes up in response to increased levels of IL-1 [79]. To the best of our knowledge, levels of cytokines measured in the subgingival fluid have never been studied in PD patients. A significant correlation has been demonstrated between the level of certain pro-inflammatory cytokines in the serum and the gingival crevicular fluid in periodontitis subjects [80]. Many studies have shown an increased peripheral and central inflammatory response in PD [81] with higher level of pro-inflammatory cytokines in the serum [82, 83], colon biopsies [27], the cerebrospinal fluid [84] and the brain [84, 85]. Inflammation has been suggested to mediate neurodegeneration [86] but the actual origin of the inflammation remains unclear. Putative « proinflammatory » Proteobacteria were more abundant in PD patients' feces than controls, whereas putative « anti-inflammatory » butyrate-producing bacteria were more abundant in controls [20]. Dysbiosis in PD could promote immune activation and systemic inflammation, which could in turn exacerbate pathogenic processes (such as triggering and maintenance of excessive α-synuclein expres-

sion) by establishing a chronic neuroinflammatory milieu.

Many studies have reported gut dysbiosis in PD patients [87]. The oral microbiota may have a great impact on the composition of gut microbiota and the health of the gastrointestinal tract [88]. Indeed, oral acid-resistant bacteria can colonize the gut through swallowed saliva. Swallowed dead oral bacteria can also be a nutritional source for gut microbiota growth (necrotrophy). Oral bacteria are poor colonizers of the healthy digestive tract [89]. However, under pathological circumstances, it has been demonstrated that oral pathogens could colonize the gut, and influence colonic composition and functions, particularly in non-alcoholic fatty liver disease, rheumatoid arthritis, and periodontitis [90–93]. In PD, no studies have specifically examined the relationship between oral and gut dysbiosis. In accordance with several studies investigating gut microbiota [19, 21, 23, 94–96], we reported an increase in the family Lactobacillaceae. An increased relative abundance of the genus *Prevotella* and Prevotellaceae family in PD buccal and sublingual mucosa samples has been reported [37]. In line with these findings, we found significantly increased levels of four bacterial species of the family Prevotellaceae (*Aloprevotella* AM420222_s, *Aloprevotella* PAC001345_s, *Prevotella* PAC001346_s and *Prevotella histicola*) in the dental plaque samples of PD patients. In saliva, the only differentially abundant Prevotellaceae species was *Prevotella shahii*, but with a higher proportion in controls. A previous study performed on stool samples showed that members of the Prevotellaceae family were found at significantly lower levels in PD patients' gut microbiota compared to controls [19]. Associations between microbial profiles and a given pathology are not necessarily expected to be the same across body locations. Differences in changes of the relative abundance of Prevotellaceae and/or their specific members, observed from different body sites when PD patients are compared to control subjects, may reflect differences in colonization of body sites by bacterial strains and species [37]. Mechanisms by which gut and oral dysbiosis are linked and may be a contributing factor to the pathophysiology of Parkinson's disease remains a subject of active research.

Our study presents several limitations. Firstly, the sample size was relatively small to draw definitive conclusions. However, participants were well examined with a detailed neurological, dental and periodontal testings. Secondly, our patient sample included early and mid-stage PD patients. We cannot exclude the possibility of the microbiota profile being different in different stages of the disease and also potentially being associated with a different prognosis. Our sample was however homogeneous in terms of the motor and the non-motor clinical signs. In addition, it has been demonstrated that gut microbiota is remarkably stable in PD patients over a 2-year period [97, 98]. Thirdly, no information about the dietary habits of our subjects were presented in our study. We cannot exclude the possibility that diet may have influenced the oral microbiome composition and inflammation level. There is still conflicting evidence regarding the relationship between dietary factors, the oral microbiome and inflammation [99–101]. Some recent studies, employing microarray and metataxonomic approaches, concluded that diet has little influence on salivary bacterial profiles [102, 103]. The primary substrates for oral bacterial growth are endogenous nutrients provided by saliva, tissue exudates, crevicular fluids, degenerating host cells, or metabolites from other bacteria [104], not directly derived from the food ingested. However, dietary intake is an important factor that influences these endogenous nutritional environments through systemic circulation. No significant difference was found in body-mass-index between study groups, arguing against major dietary differences. Given the assortment of potential health effects of oral microbiome, an increase in knowledge concerning how host lifestyle factors influence human oral microbial composition is warranted. Fourthly, most of our patients were taking dopamine replacement therapy and we did not have adequate representation of unmedicated subjects to fully evaluate the consequences of treatment on the outcomes. Fifthly, while our study focused on the taxonomic composition of the microbiota, PD might be associated with changes in microbial metabolic pathways that can be investigated by means of metagenomics, metatranscriptomics, metaproteomics and metabolomics. However, the use of 16S rRNA-gene based metataxonomic approach made comparisons with previously published data easier, since the majority of published studies used this method. Finally, we only assessed the oral microbiome on a single occasion, and it would be interesting to follow patients over time in order to investigate the influence of PD progression on microbiota composition.

In conclusion, our findings suggest that the oral microbiome is altered in PD. Oral inflammation is present in PD and probably precedes poor oral health. Additional studies are warranted to further elucidate

the causal relationships between oral dysbiosis and the pathogenesis and clinical manifestations of PD, as well as the suitability of an analysis of oral microbiota as a potential biomarker or therapeutic target for PD.

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CONFLICT OF INTEREST

The authors have no financial disclosures and no conflicts of interest concerning the research related to the manuscript.

SUPPLEMENTARY MATERIAL

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