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Oral Microbiota and Porphyromonas Gingivalis Kgp Genotypes Altered in Parkinson's Disease with Mild Cognitive Impairment

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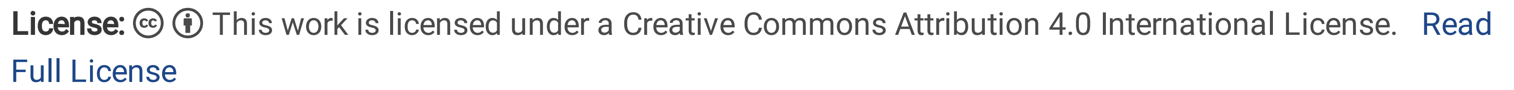
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Abstract

Cognitive impairment (CI) is a common complication of the non-motor symptoms in Parkinson's disease (PD), including PD with mild cognitive impairment (PD-MCI) and PD dementia. Recent studies reported the oral dysbiosis in PD and CI, respectively. *Porphyromonas gingivalis* (*P. gingivalis*), a pathogen of oral dysbiosis, play an important role in PD, whose lysine-gingipain (Kgp) could lead to AD-type pathologies. No previous study investigated the composition of oral microbiota and role of *P. gingivalis*in PD-MCI. This study aimed to investigates the differences of oral microbiota composition, *P. gingivalis* copy number, and Kgp genotypes among PD-MCI, PD with normal cognition(PD-NC) and periodontal status-matched control (PC) groups. The oral bacteria composition, the copy number of *P. gingivalis*and the Kgp genotypes in gingival crevicular fluid from PD-MCI, PD-NC, and PC were analyzed using 16S ribosomal RNA sequencing, quantitative real-time PCR, and Mse restriction. We found that the structures of oral microbiota in PD-MCI group were significantly different compared to that in PD-NC and PC group. The relative abundances of *Prevotella*, *Lactobacillus*, *Megasphaera*, *Atopobium* and *Howardella* were negatively correlated with cognitive score. Moreover, there was a significant difference of Kgp genotypes among the three groups. The predominant Kgp genotypes of *P. gingivalis* in the PD-MCI group was primarily Kgp , whereas in the PD-NC group was mainly Kgp . The Kgp correlated with lower MMSE and MoCA scores, suggested that PD-CI may be related to Kgp genotypes .

Introduction

Parkinson's disease (PD) is a prevalent neurodegenerative disease caused by the death of dopaminergic neurons in the substantia nigra pars compacta (SNpc). Cognitive impairment (CI) is a common complication of the non-motor symptoms in PD, including PD with mild cognitive impairment (PD-MCI) and PD dementia (PDD). PD-MCI, an independent risk factor for PDD [1]. is a transitional state that does not fulfill the diagnostic criteria for PDD. A 5-year follow-up study of PD patients revealed that the cumulative incidence of PD-MCI in patients aged ≥ 65 years was 41.3% after a span of 5 years, and the conversion rate of PD-MCI patients progressing to PDD within 5 years was approximately 39%-50% [2].

The pathogenesis of PD-CI is not absolutely clarified. Recent studies have found that related pathologies of PD-CI include deposition of α-Synuclein (α-Syn), Alzheimer's disease(AD)-type pathologies (amyloid-β, tau and neurofibrillary tangles) and neuroinflammation [3, 4]. Numerous studies have reported the presence of oral dysbiosis in PD and AD patients [5–8]. *Porphyromonas gingivalis* (*P. gingivalis*), as a pathogen of oral dysbiosis, has attracted much attention in recent studies. *P. gingivalis* played a crucial role in PD [9–12] and AD [13–19]. lysine-gingipain (Kgp), one of major virulence factors in *P. gingivalis*, could lead to AD-type pathologies [19]. According to Beikler et al., the gene encoding Kgp could be identified into two genotypes, Kgp and Kgp [20].

Nonetheless, no previous study investigated the composition of oral microbiota and role of *P. gingivalis* in PD-MCI. Our previous review suggested that *P. gingivalis* possibly play an important part in PD-CI development [21]. This study aimed to investigates the differences of oral microbiota, especially *P.*

gingivalis, as well as whether Kgp genotypes was different among PD-MCI, PD patient with normal cognition(PD-NC) and periodontal status-matched control(PC) groups.

Methods

Recruitment

This study was approved by the Ethics Committee of Maoming People's Hospital and informed consents were obtained from the participants. The PD patients were recruited and assessed at the Department of Neurology in Maoming People's Hospital, Guangdong Province, China (from January 2023 to July 2023). All patients recruited for this study were diagnosed for PD according to the UK Brain Bank criteria [22]. According to the PD-MCI diagnostic criteria published by MDS in 2012 [23], cognitive assessment was conducted for each patient across five cognitive domains, including attention and working memory (Digit Span Backward and Digit Symbol Test), executive function (10 points Clock Drawing Test and Verbal Fluency Test), language (Wechsler Adult Intelligence Scale-IV Similarities and Boston Naming Test), memory (Wechsler Memory Scale-IV: visual recognition and visual reproduction), and visuospatial function (Wechsler Adult Intelligence Scale-IV: object assembly and block test). Of these patients, 15 were clinically diagnosed with PD-NC and 20 with PD-MCI. 15 age-, sex-, and periodontal status-matched subjects were enrolled as PC.

Clinical assessment

The clinical data were obtained via face-to-face interviews with movement disorder specialists. The weight and height of each participant were measured, and the body mass index (BMI) was subsequently calculated. PD clinical characteristics included disease duration, education(years), motor and non-motor symptoms, and medication. The part III scores of MDS-Unified Parkinson's Disease Rating Scale (MDS-UPDRS) and Hoehn and Yahr stage (H-Y stage) were analyzed during the "on" state. The PD-related non-motor symptoms were evaluated using the Parkinson's Disease Questionnaire (PDQ-39). Dental and periodontal parameters were assessed using the Kayser-Jones brief oral health status examination (BOHSE), plaque index and percentage of presence of bleeding on probing (BOP+). Cognition abilities were assessed using the Mini-Mental State Examination (MMSE) and Montreal Cognitive Assessment (MoCA), and the scores were obtained from two other neuropsychological tests in each of the five cognitive domains. On the day of the samples collection, all the participants completed a questionnaire assessing their dietary habits in the last month, including the consumption of coffeine and alcohol.

Samples collection of gingival crevicular fluid

Participants were asked to avoid teeth brushing in the morning of the sampling. For samples collection of gingival crevicular fluid(GCF)[24], after gargling with water, supragingival plaque was removed from the collection sites, which were then isolated with cotton rolls and dried by air blowing. A sterile paper point was then placed at the orifice of the periodontal pocket for 30s. GCF was collected from the mesio-buccal sites of periodontal sulci of the first molar in each quadrant. Four sterile paper points were therefore generated for each participant unless they had no first premolar in one or several quadrants. Within the

following 3 hours, samples were transferred for storage and kept at a temperature of − 80℃ until further processing.

GCF DNA extraction and 16S rRNA genes sequencing

The DNA in GCF was extracted using a QIAamp DNA Stool Mini Kit (Qiagen, Hilden, Germany). Polymerase chain reaction (PCR) amplification of 16S rRNA genes was performed with general bacterial primers (357F 5'-ACTCCTACGGRAGGCAGCAG-3' and 806R 5'-GGACTACHVGGGTWTCTAAT-3'). Prior to library pooling, the barcoded PCR products were purified using a DNA gel extraction kit (Axygen, China) and quantified using the FTC-3000 real-time PCR. The 16S rRNA amplicon (V3–V4 regions) sequencing analysis was performed using an Illumina novaseq6000 2×250bp. The sequences were subjected to quality control and subsequently analyzed using the software mentioned below. The 16S sequences were analyzed using a combination of mothur (version 1.33.3), UPARSE (usearch version v8.1.1756), and R software (version 3.2.3). The demultiplexed reads were clustered at 97% sequence identity into operational taxonomic units (OTUs) using the UPARSE pipeline. The OTU representative sequences were selected and their taxonomies were assigned against the Silva 128 database with a confidence score ≥ 0.6 using the classify.seqs command in mothur.

Quantitative real-time PCR

Quantitative real-time PCR (qPCR) was conducted using a Hongshi SLAN-96S real-time PCR system. Copy number of the *P. gingivalis* genome in GCF samples was determined by qPCR. Amplification was performed in a 25µL final volume containing 5µL of template DNA, 12.5 µL of SRBR Premix Ex Taq (Probe qPCR; Takara), 0.5µL each of 10µM forward (ACCTTACCCGGGATTGAAATG) and reverse primers (CAACCATGCAGCACCTACATAGAA), and 1µL of a 10µM probe (ATGACTGATGGTGAAAACCGTCTTCCCTTC). All qPCR reactions were run with 3 replicates per DNA. PCR amplification was performed using the following cycling parameters: an initial denaturation step at 95°C for 30 seconds followed by 45 cycles at 95°C for 10 seconds and at 60°C for 60 seconds. Copy number was determined from the standard curve generated using a synthetic template.Standard curves were set up by serially diluting plasmid of a pUC57 vector with the appropriate insert from 107 to 101 target gene copies/µl. The standard curve was obtained using linear regression of threshold cycle numbers versus log copy numbers of target plasmid.

Analysis of P. gingivals Kgp genotypes

Detection of Kgp and Kgp by PCR amplification and MseI restriction [20]. The PCR reaction mixture contained 10µL 5xBuffer, 1µL each of 10µM Kgp forward (GAACTGACGAACATCATTG) and reverse primers (GCTGGCATTAGCAACACCTG), 1µL of 10mM dNTP, 1U of Phusion hyperfidelity DNA polymerase and 5ng-50ng of template. PCR amplification was performed using the following cycling parameters: 2min at 94°C followed by 40 cycles 30s at 94°C, 30 s at 60°C and 60s at 72℃. According to the method from Beikler et al.[20], the PCR products were digested by MseI, and the target fragment of Kgp was 447bp + 288bp + 135bp and Kgp was 870bp.

Bioinformatic and statistical analysis

The SPSS (version 26.0, SPSS Inc., IL, USA) and R software (version 3.2.3, the R Project for Statistical Computing) were used for the statistical analysis of data. The Shapiro-Wilk test was employed to assess the normality test. Clinical dates from three groups were compared using the one-way ANOVA or Kruskal-Wallis H test for quantitative variables, and Pearson's chi–square test for categorical variables. The differences between PD-NC and PD-MCI groups were compared using the t-test (PDQ-39 and MDS-UPDRS III) or Wilcoxon rank (duration and H-Y stage) for quantitative variables and Pearson's chi-square test for categorical variables, with alpha set at 0.05. The alpha-diversity metrics (including Chao, Shannon, Simpson, sobs indexes, and others) as well as the beta-diversity metrics (unweighted UniFrac ANOSIM indexes, weighted UniFrac ANOSIM indexes, and PERMANOVA analysis) were calculated using Quantitative Insights into Microbial Ecology. Alpha-and beta-diversity analyses were performed using mothur software, and results were visualized through principal coordinate analysis. Differences in abundance across multiple taxonomic levels among the three groups were detected using a Kruskal–Wallis test. The linear discriminant analysis (LDA) effect size method was utilized to characterize the taxa with statistical significance and biological relevance. The datas from qPCR were compared using Wilcoxon rank, and P. gingivals Kgp genotypes among three groups were compared using Pearson's chi–square test. The post hoc Bonferroni adjustments were applied to account for multiple comparisons, with alpha set at 0.0167.

Results

Clinical date

A total of 50 participants were recruited for the study, including 20 PD-MCI patients, 15 PD-NC patients and 15 healthy controls. Both the patients and the controls reported to be omnivores with a conventional diverse diet and without any dietary restrictions. Demographic and clinical parameters of each group are shown in Table 1. There were no significant differences among the three groups in age, sex, BMI, and education. Furthermore, no significant differences in duration, MDS-UPDRS III score, H-Y stage, use of anti-Parkinson medicine and PDQ-39 were found between the PD-NC and PD-MCI. As expected, PD-MCI group had significantly lower MMSE and MoCA scores compared PD-NC ($P < 0.001$) and PC ($P < 0.001$) groups, while no difference was found between the PD-NC and PC groups in MMSE and MoCA scores. Since almost all PD patients had varying degrees of periodontal disease, we included controls matched for periodontal status. There were no significant differences in periodontal parameters (BOHSE, plaque index and BOP+) among the three groups. In addition, there were no significant differences in alcohol and caffeine intake among the three groups.

Table 1
Selected demographic and clinical parameters of PC group, PD-MCI group and PD-NC group

		PC (n = 15)	PD-MCI (n = 20)	PD-NC (n = 15)	P-value	P_{corr} (Bonferroni corrected)		
						PD-MCI VS.PD-NC	PD-MCI VS. PC	PD-NC VS. PC
Age[A]		61.80(7.60)	61.60(7.46)	61.27(7.59)	0.981	0.898	0.938	0.847
Sex[C]	F	5	8	6	0.906	1.000	0.686	0.705
	M	10	12	9				
BMI[A]		22.22(1.75)	21.75(1.68)	21.46(1.88)	0.495	0.640	0.433	0.244
Education[A]		10.73(4.59)	8.90(3.64)	10.40(4.14)	0.368	0.288	0.196	0.824
Duration[W]			3.55(2.69)	3.33(2.35)	0.892			
PDQ-39[T]			44.10(12.45)	47.47(13.34)	0.788			
MMSE[K]		29.07(0.80)	26.00(1.62)	29.07(1.28)	< 0.001	< 0.001	< 0.001	> 1.000
MoCA[K]		28.00(1.60)	21.45(1.99)	27.07(1.39)	< 0.001	< 0.001	< 0.001	0.991
H-Y stage[W]			1.35(0.59)	1.33(0.49)	0.862			
MDS-UPDRS III[T]			27.80(8.28)	24.67(8.58)	0.946			
BOHSE[A]		10.33(3.64)	10.05(2.89)	10.93(3.41)	0.732	0.435	0.802	0.619
Plaque Index[A]		1.30(0.45)	1.61(0.47)	1.55(0.57)	0.179	0.690	0.072	0.185
BOP+%[A]		51.70(16.24)	55.49(15.24)	61.18(20.00)	0.318	0.334	0.519	0.135
Anti-Parkinson medicine[C]	Y		16	14	0.365			

Date are expressed as mean (SD)

Pcorr denotes values corrected for multiple comparisons using the Bonferroni method. Alpha was set at 0.0167.

[A]maens with One-way ANOVA, [C]maens with Chi-square test, [W]maens with Wilcoxon rank, [K]maens with Kruskal-Wallis H test, [T]maens with Student’s t-test. Alpha was set at 0.05.

		PC (n = 15)	PD-MCI (n = 20)	PD-NC (n = 15)	P-value	P_{corr} (Bonferroni corrected)		
						PD-MCI VS.PD-NC	PD-MCI VS. PC	PD-NC VS. PC
	N		4	1				
COMT-inhibitors[C]	Y		3	2	> 1.000			
	N		17	13				
Alcohol[C]	Y	5	4	4	0.664			
	N	10	16	11				
Coffeine[C]	Y	5	5	2	0.500			
	N	10	15	13				
Date are expressed as mean (SD)								
Pcorr denotes values corrected for multiple comparisons using the Bonferroni method. Alpha was set at 0.0167.								
[A]maens with One-way ANOVA, [C]maens with Chi-square test, [W]maens with Wilcoxon rank, [K]maens with Kruskal-Wallis H test, [T]maens with Student’s t-test. Alpha was set at 0.05.								

Sequence data analysis

On average 70,034 reads were sequenced per sample, 67,349 of which were assigned to Operational Taxonomic Units (OTUs). Sequence counts per sample varied from 60,970 (PC-8) to 72,353 (PD-NC-1) with a mean value of 67,349. In total, 956 distinct OTUs were identified across the 50 samples. The full dataset included bacteria from 221 genera, 95 families, 59 orders, 30 classes, and 23 phyla. As for oral microbiota, no significant differences of the mean community alpha-diversity indexes were found among the three groups (including Chao, Simpson, Shannon, and PD_whole_tree) (Suppl. Figure 1). Statistical discrepancies were found in beta-diversity base in the unweighted UniFrac ANOSIM metric (qualitative, ANOSIM R = 0.071, P = 0.028), as well as the weighted UniFrac ANOSIM metric (quantitative, ANOSIM R = 0.090, P = 0.017) among the PD-MCI, PD-NC, and PC groups (Fig. 1A). Moreover, according to the the UniFrac index, the structures of oral microbiota in PD-MCI group exhibited significant dissimilarities compared to that in PD-NC and PC group, respectively (Fig. 1B, C), whereas no significant differences were observed between the PC group and PD-NC group.

Alteration of oral microbiota

Linear discriminant analysis (LDA) with effect size (LEfSe) is commonly employed to identify the presence and effect size of region-specific OTUs among different groups (Suppl. Figure 2). Base on the LDA LEfSe, significant differences were observed in oral microbiota among the PD-MCI, PD-NC and PC groups. The relative abundance of genera Prevotella, Lactobacillus, Megasphaera, Atopobium, Howardella was higher in the PD-MCI group compared with the PD-NC and PC groups. The abundances of *Prevotella* (MMSE: r=-0.452, p = 0.001; MoCA: r=-0.413; p = 0.003), *Lactobacillus* (MMSE: r=-0.332, p = 0.019; MoCA: r=-0.333; p = 0.018), *Megasphaera* (MMSE: r=-0.351, p = 0.013; MoCA: r=-0.426; p = 0.002), *Atopobium* (MMSE: r=-0.510, p = 0.002; MoCA: r=-0.367; p = 0.009) and *Howardella* (MMSE: r=-0.459, p < 0.001; MoCA: r=-0.367; p = 0.009) were negatively correlated with MMSE and MoCA scores (Suppl. Figure 3). Additionally, a higher relative abundance of the genera *Porphyromonas*, *Aggregatibacter*, *Corynebacterium*, *Enhydrobacter*, *Curvibacter*, *Micrococcus* and *Caulobacter* was found in PD-NC compared to the other two groups. Finally, the PC group exhibited a higher relative abundance of 17 genera in comparison to the other two groups. At the species level, no statistically significant difference in the relative abundance of *P. gingivalis* among the three groups.

Predictive function analysis

PICRUSt based on closed-reference OTU was used to predict the abundances of functional categories in the KEGG ortholog (KO). In this study, 1633 KOs having significantly different abundances were identified in GCF samples among PD-MCI, PD-NC and PC. A plot of top 20 KOs identified with significantly different abundances in the oral microbiota among the three groups (FDR, P < 0.05) was made. The microbial gene functions related to purine metabolism, pyrimidine metabolism, peptidoglycan biosynthesis, citrate cycle (TCA cycle), amino acid metabolism (glycine, serine, threonine, alanine, aspartate and glutamate), one carbon pool by folate, protein export, beta-Lactam resistance, photosynthesis and antifolate resistance in the level 3 KEGG pathway were higher in the oral microbiome of the PD-MCI group. However, the microbial gene functions related to quorum sensing, fatty acid biosynthesis, propanoate metabolism, biotin metabolism, sulfur relay system in the level 3 KEGG pathway were lower in the oral microbiome of the PD-MCI group. Additionally, the microbial gene functions related to phosphotransferase system (PTS) in the level 3 KEGG pathway were higher in the fecal microbiome of the PD-NC group (Suppl. Figure 4).

Copy number of P. gingivalis

The quantification of *P. gingivalis* DNA copy number in the GCF was performed using qPCR. No statistically significant differences were observed in the copy number of *P. gingivalis* DNA among the three groups (P = 0.718). Furthermore, there was also no significant difference between PC and PD (PD-MCI + PD-NC) groups (P = 0.863) (Suppl. Figure 5).

Kgp genotypes of P. gingivalis

The results are presented in Fig. 2, where samples 1 to 15 represent the PC group, samples 16 to 35 correspond to the PD-MCI group, and samples 36 to 50 pertain to the PD-NC group. The results of all the successfully detected samples were as follows: in the PC group, 6 samples were Kgp and 4 samples were Kgp ; in the PD-MCI group, 7 samples were Kgp and 11 samples were Kgp ; in the PD-NC, 10 samples

were Kgp and 1 sample was Kgp . There were significant differences in Kgp genotypes among the PD-MCI, PD-NC, and PC groups (p = 0.022). After post hoc pairwise comparisons, there was a significant difference of Kgp genotypes between PD-MCI and PD-NC groups (P = 0.008). The predominant Kgp genotype of *P. gingivalis* in the PD-MCI group was primarily Kgp , whereas the dominant Kgp genotype in the PD-NC group was mainly Kgp . Moreover, the Kgp of *P. gingivalis* correlated with lower MMSE(P = 0.044) and MoCA (P = 0.007) scores.

Discussion

Previous study indicated that the oral microbiota of patients with PD differed from those of PC[5–7]. The oral microbiota composition differences among PD-MCI and PD-NC have not been investigated in previous studies. This study was novel in further showing that the oral microbiota exhibited significant alterations in the PD-MCI group compared to both the PD-NC and PC groups. Although no statistically significant differences were found in alpha-diversity indices among PC, PD-MCI and PD-NC groups, this study confirmed the significant differences in beta-diversity indices, particularly at the family and genus level, between the PD-MCI and PD-NC groups, and between the PD-MCI and PC, respectively. These results provided evidence that the oral microbiota in PD-MCI were different from those of PD-NC and PC.

In this study, we observed a significant increase in the relative abundance of *Prevotella*, *Lactobacillus*, *Megasphaera*, *Atopobium* and *Howardella* in GCF of PD-MCI group. Furthermore, the abundance of these genera exhibited a negative correlation with MMSE and MoCA scores. Similar to our finding, previous studies on microbiota also showed the crucial roles of these genera in PD and AD. First, an increased abundance of *Prevotella* was commonly found in oral microbiota in recent studies. Yay et al. revealed that many species of *Prevotella* were more prevalent in subgingival microbiome of PD [7], which was in line with the outcomes obtained from buccal and sublingual mucosa[5] and dental plaque in early researches[6]. In addition, *Prevotella* abundance was also higher and was negatively correlated with cognitive impairment in the gut microbiota of patients with MCI and AD [8, 25, 26]. However, several previous studies showed that members of the *Prevotellaceae* family were found at significantly lower levels in PD patients' gut microbiota [27–29]. In our previous study on intestinal flora [30], no significant difference in the abundance of *Prevotella* was found among PC, PD-MCI and PD-NC groups. These findings indicated that increased abundance of *Prevotella* in the oral cavity was related with PD, while increased abundance in the gut is associated with cognitive impairment, reflecting bacterial colonization may play distinct roles in different body sites. Second, previous researches revealed that *Lactobacillus* had higher relative abundance in oral microbiome of PD patients [6]. The increased abundances of *Lactobacillus* and *Megasphaera* in the gut correlated with worse motor and cognitive function in patients with PD [31]. In addition, previous studies showed that dysregulation of amino acid homeostasis induced by *Lactobacillus* could contribute to AD pathogenesis [32], and *Lactobacillus* altered glutamate metabolism, which modulated GABA levels to induce neural dysfunction [33]. In this study, the functional interpretation of the oral microbiome demonstrated that the amino acid (including glycine, serine, threonine, alanine, aspartate and glutamate) metabolism was higher in PD-MCI group. This dysregulation of amino acid metabolism may be associated with the higher relative abundance of *Lactobacillus* that we detected in the PD-MCI

group. Finally, a recent study by Na et al. also showed that increase in the relative abundance of *Atopobium* were observed in the AD group compared with cognitively unimpaired periodontitis patients [8].

The currently available studies underlined the association between *P. gingivalis* and PD, and between *P. gingivalis* and cognitive impairment, respectively. A recent animal study by Feng et al. showed that, orally administrating live *P. gingivalis* to Mouse models of PD can induce an increase in the accumulation of α-Syn in the colon neurons and a reduction of dopaminergic neurons in the substantia nigra [12]. Dominy et al. confirmed that Kgp could lead to AD-type pathologies, and Kgp inhibitor treated *P. gingivalis* brain infection and prevented loss of hippocampal interneurons [19]. Nevertheless, the present study showed that the abundance of genus *Porphyromonas* was higher in the patients with PD-NC compared with that in PD-MCI. And similar with the results from Na et al. [8], no significant differences were observed in the relative abundance and copy number of *P. gingivalis* among the three groups. According to sequence differences in the region encoding the catalytic domain, Beikler et al. identified Kgp genotype into Kgp and Kgp by PCR amplification and Mse -mediated restriction [20]. No significant differences in periodontal parameters between two Kgp genotypes in our study was partially consistent with Beikler et al., who concluded no significant differences between Kgp and Kgp with respect to the enzymes activity of Kgp and the pathogenicity of periodontal disease. The contribution of these two Kgp genotypes to the pathogenesis of neurodegenerative disorders remains unexplored in prior studies. Interestingly, we found a significant disparity in Kgp genotypes between the PD-MCI and PD-NC groups, with a higher prevalence of the Kgp in the PD-MCI group, while the Kgp predominated in the PC-NC group. Furthermore, the Kgp was correlated with lower MMSE and MoCA scores. These results implied the existence of potential differences in neurotoxicity between these two Kgp genotypes, indicating that the cognitive impairment induced by *P. gingivalis* may not be influenced by its abundance or concentration, but rather by the Kgp genotypes. To the best of our knowledge, no study has investigated the different roles of these two Kgp genotypes in cognitive impairment. The identification of these two Kgp genotypes holds potential as biomarkers, thereby presenting a novel diagnostic and therapeutic target of cognitive decline in PD. However, further studies are warranted to validate this hypothesis.

A strength of this study was the recruitment of periodontal status-matched healthy subjects as controls, which means that analysis of oral microbiota among three groups were performed after controlling for a possible confounding factor, the degree of periodontosis. Moreover, the individuals enrolled were all Cantonese people with a balanced diet. However, our study presents several limitations that warrant consideration. Firstly, considering the potential bias arising from limited sample size, it is crucial to conduct large-scale studies in order to validate the findings of this study. Secondly, the majority of our patients were undergoing dopamine replacement therapy, which limited the representation of unmedicated subjects and hindered a comprehensive evaluation of treatment outcomes. Additionally, this study could not provide evidence for a causal relationship between oral dysbiosis and PD-CI. Finally, we only assessed the Kgp genotpye on a single occasion for normal cognition and mild cognitive impairment in PD. Kgp genotypes may be different in different stages of cognitive impairment in PD. The conversion of Kgp to Kgp may occur during the progression of cognitive decline. Thus, it would be necessary to follow patients over time in order to investigate the impact of Kgp genotypes on cognitive function patients with PD.

In conclusion, our study revealed that composition of oral microbiota in PD-MCI group was significant different compared with PC and PD-NC groups. Furthermore, we found that Kgp genotype of *P. gingivalis* identified by Mse restriction is related with PD-MCI and lower cognitive scores. The Kgp may be a new biomarker for PD-CI.

Declarations

Funding

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Competing Interests

The authors have no relevant financial or non-financial interests to disclose.

Author Contributions

Xiong Zhang and Renshi Xu conceptualized and designed the study. Dongcheng Li, Tengzhu Ren and Hao Li organize the researches. Dongcheng Li, Mingdi Huang, Jiaxin Chen and Qishan He performed data collection and analysis. Dongcheng Li, Qishan He and Tengzhu Ren analyzed and interpreted data. Dongcheng Li wrote the manuscript draft. Xiong Zhang and Tengzhu Ren contributed to the drafting and critical revision of the manuscript. All authors read and approved the final manuscript.

Data Availability

Raw sequencing data was deposited in the NCBI GenBank under BioProject ID PRJNA1030740.

Ethics Approval

The studies involving human participants were reviewed and approved by Ethics Committee of Maoming People's Hospital (reference No. PJ2022MI-K011-01).

Consent to Participate

Informed consent was obtained from all individual participants included in the study.

Consent for Publication

Not applicable

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Figures

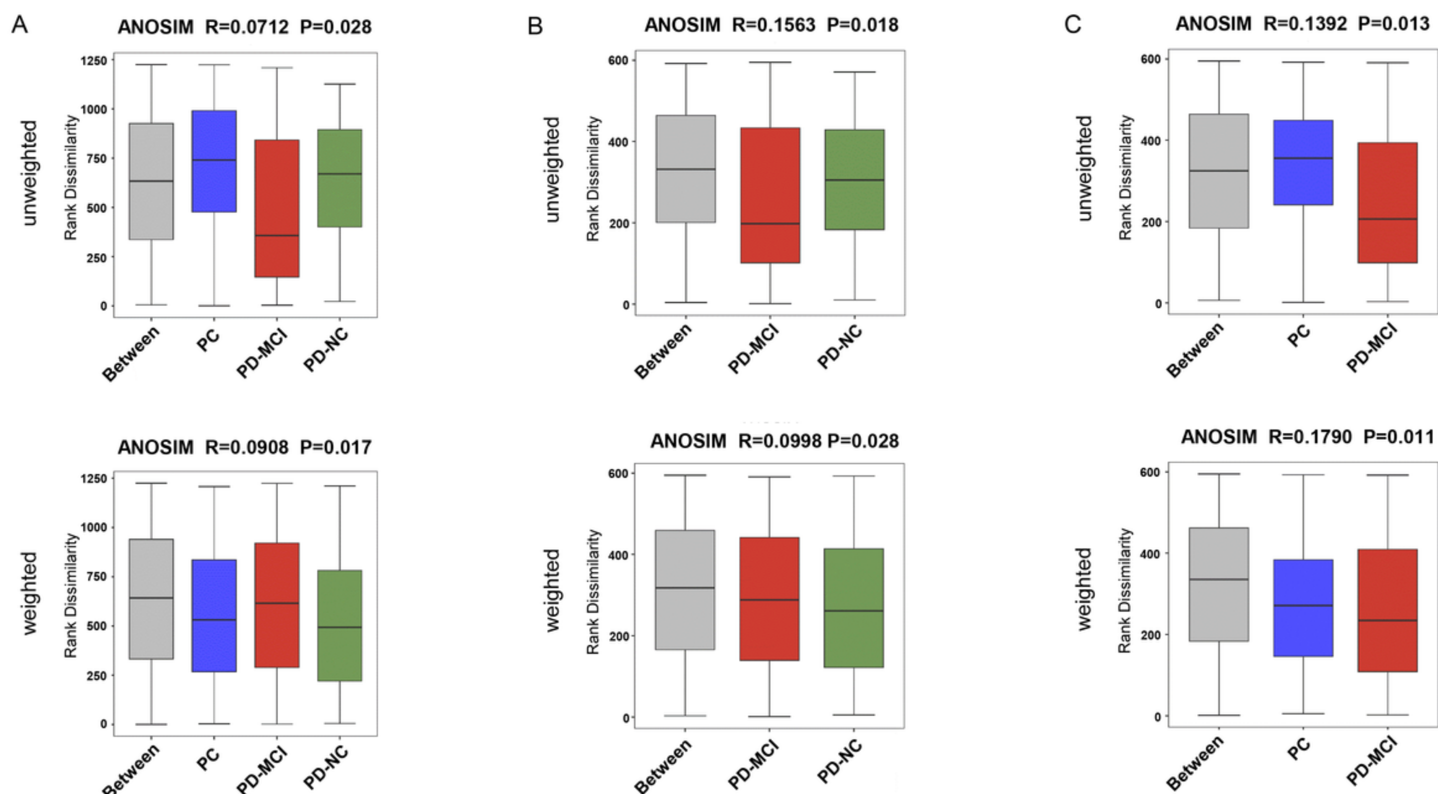

Figure 1

The beta-diversity indices of the oral microbiome in the PD-MCI, PD-NC, and health group. **a)** Unweighted and weighted ANOSIMs Unifrac analysis based on the distance matrix of UniFrac dissimilarity of the oral microbial communities in the three groups. Respective ANOSIM R values show the community variation between three groups and significant P values are indicated. The axes represent the two dimensions explaining the greatest proportion proportion of variance in the communities. **b)** Unweighted and weighted ANOSIMs Unifrac analysis between PD-MCI and PD-NC groups. **c)** Unweighted and weighted ANOSIMs Unifrac analysis between PC and PD-MCI groups. ANOSIM, analyses of similarities.

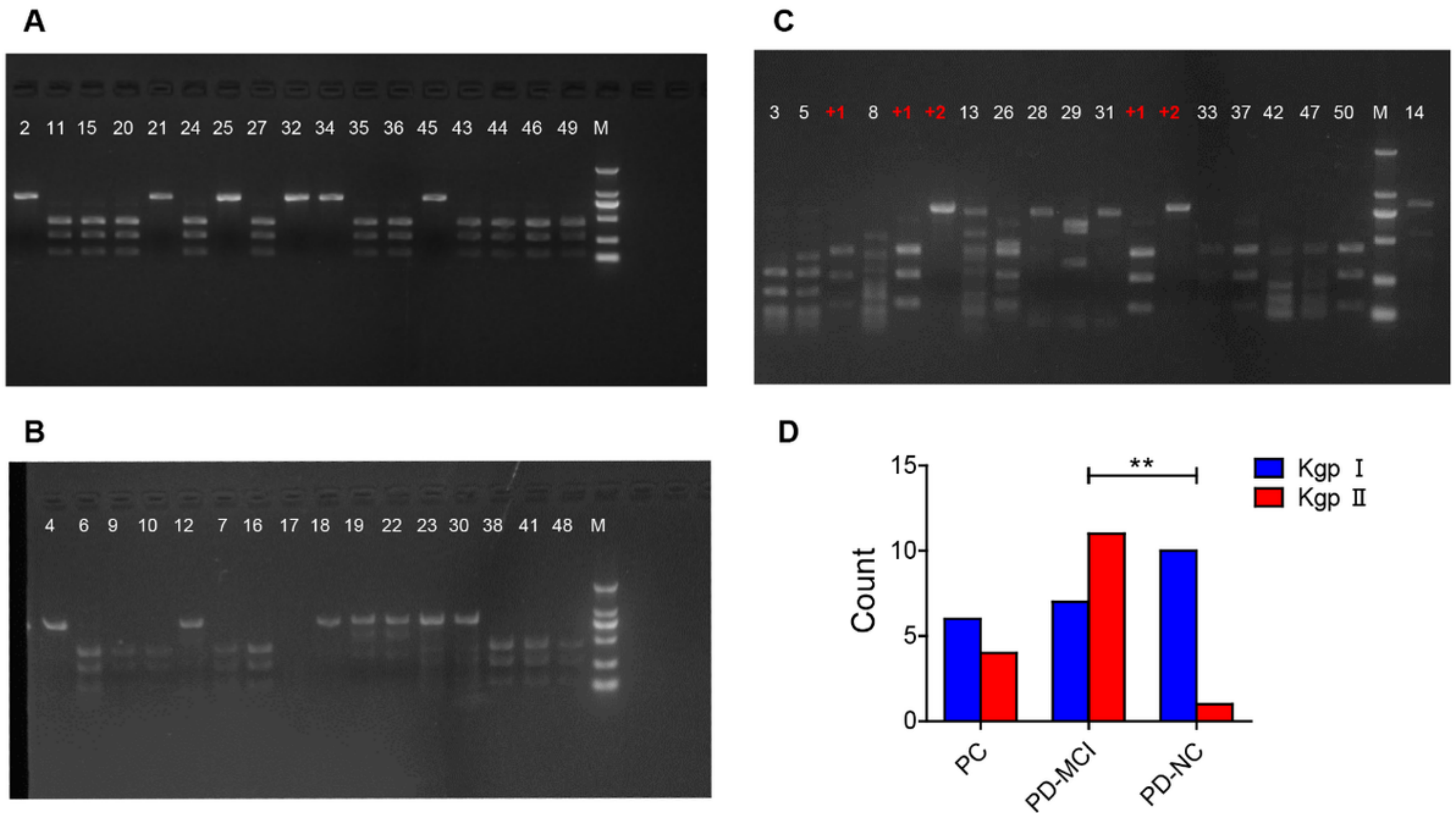

Figure 2

The results of *Porphyromonas gingivals* Kgp genotypes analysis in gingival crevicular fluid in the three groups. **a) b) c)** The results of Mse restriction were displayed by gel electrophoresis. Samples 1 to 15 is the PC group, 16 to 35 is the PD-MCI group, and 36 to 50 is the PD-NC group. The absence of certain samples in the figure due to experimental failures (such as sample 1, 39 and 40). Samples +1 and +2 marked in red represent control samples for Kgp I and Kgp II, respectively. The enzyme-digested products of some samples did not meet the expectation (sample 3, 5, 8, 13, 17, 29, 42 and 47), which were excluded from the statistical analysis. **d)** The count of different Kgp genotypes in each group. There was a significant difference of Kgp genotypes between PD-MCI and PD-NC groups. **P<0.01.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- ESM1.pdf
- ESM2.xlsx