Analysis of oral microbiome in chronic periodontitis with Alzheimer's disease: Pilot study

Hee Sam Na
Pusan National University School of Dentistry
Na-Yeon Jung
Pusan National University School of Medicine
Suji Choi
Pusan National University School of Medicine
Si yeong Kim
Pusan National University School of Dentistry
Hyun-Joo Kim
Pusan National University School of Dentistry
Ju Youn Lee
Pusan National University School of Dentistry
Jeomil Choi
Pusan National University School of Dentistry
Yun Hak Kim
Pusan National University School of Medicine
Jae-Hyung Lee
Kyung Hee University School of Dentistry
Jin Chung (jchung@pusan.ac.kr)
https://orcid.org/0000-0002-6859-615X

Research

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Abstract

Background

Alzheimer's disease (AD) dementia is the most common form of dementia in the elderly. Chronic periodontitis (CP) is a progressive destructive disease in the periodontal tissues, which is also common in the elderly. CP is known to be associated with an increase in cognitive decline in Alzheimer's disease (AD). Recently, a potential role for pathogenic microbes in the development or exacerbation of AD pathology has been proposed. To reveal the association between periodontitis-related microbes and AD, we investigated the oral microbiome in AD patients with CP.

Methods

Fifteen AD dementia (AD) with CP and 14 cognitively unimpaired (CU) participants with CP were recruited. Buccal, supragingival and subgingival plaque samples were collected with the full-mouth periodontal examination. Alpha diversity, beta diversity, LEfSe (linear discriminant analysis effect size), metabolic pathway prediction and network analysis were applied to compare the microbiome features.

Results

All participants had moderate to severe chronic periodontitis. The level of alpha diversity in subgingival microbiota of the AD group was higher than the CU group. Also, principle coordinate analysis showed significant difference in subgingival samples. When significant taxa were analyzed by LEfSe, various *Prevotella* spp. were more prevalent in subgingival samples from AD group. Furthermore, subgingival microbiome network analysis showed distinctive network complexity in AD compared to CU group.

Conclusion

We found that subgingival microbiome of AD patients had increased microbial diversity. The composition of subgingival microbiome was different between the AD and the CU groups. This pilot study provides a novel view at the changes of subgingival microbiome in AD patients with CP. Our findings need further well-designed studies with adequate sample size to confirm oral microbiome characteristics in AD with CP.

Introduction

According to the 2019 World Alzheimer Report, there are 50 million people living with dementia worldwide. Alzheimer's disease (AD) dementia is the most common form of dementia. It is characterized by progressive cognitive dysfunction and abnormal behavior (1). AD is a multifactorial disease primarily caused by genetic, aging, and environmental factors (2–4). Accumulating evidence indicates that

peripheral infections and oxidative stress may aggravate inflammation in the brain and play an important role in the pathogenesis of AD (5). Cerebrovascular disease (6), diabetes mellitus (7), hypertension (8), hyperlipidaemia (9) and periodontitis (10) have been reported to be associated with AD.

Oral hygiene is an increasingly recognized environmental factor and is significantly compromised in patients affected by AD because the cognitive processes of learning, attention and memory are progressively damaged. Tooth loss and poor oral hygiene are recognized as an important risk factor for dementia (11, 12). Chronic periodontitis (CP) is a chronic inflammation of the tissue surrounding the teeth induced by complex bacteria interactions with host cells, resulting in breakdown and loss of supporting structures around the teeth. CP has been suggested to be associated with AD and cognitive decline (10, 13, 14). In a Taiwan cohort study, 10-year CP exposure was associated with a 1.7-fold increase in the risk of developing AD (10). The mechanism for the relationship between CP and AD remains unclear, but there are evidences to support that systemic inflammation related to CP can be associated with cognitive impairment (14, 15).

Beside oral disease, microbiome is in the spotlight in neurodegenerative diseases. Gut microbes have been found to be associated with AD both in animal models and in patients (16, 17). Interestingly, oral bacteria have been frequently observed in the AD brain tissue compared with normal controls suggesting possible roles of oral microbiome in pathogenesis of AD (18, 19). Recently, salivary microbiome in AD patients showed reduced diversity compared with healthy controls (20). Oronasal cavity is close to brain and has associated with the brain via the trigeminal and olfactory nerves (21). Thus, dysbiotic oral microbiome has been suggested to increase the infection of opportunistic pathogens in the brain of AD patients and further contribute to the development of AD (18, 22). Few studies have been conducted to characterize the oral microbiome in AD (20). Moreover, there is no study that has focused on CP patients with AD. In this study, we compared the oral microbiome in CP with or without AD to characterize the oral microbiome that is related with AD.

Material And Methods

Study Population

AD patients were recruited at the dementia clinic at Pusan National University Yangsan Hospital (PNUYH) between March 2018 and September 2018. All patients were evaluated with comprehensive interviews, neurological examinations, and neuropsychological assessments. Blood tests to exclude secondary causes of dementia included a complete blood count, blood chemistry tests, thyroid function tests, vitamin B12/folate, and syphilis serology. Conventional brain MRI or CT scans confirmed the absence of structural lesions such as brain tumors, traumatic brain injuries, hydrocephalus, or severe white matter diseases. AD patients fulfilled the NIA-AA core clinical criteria for probable AD dementia (1). All AD patients were assessed by one dental practitioner and revealed having periodontal disease.

Cognitively unimpaired (CU) people were enrolled upon being scheduled to undergo periodontal treatment at the Department of Periodontics of Pusan National University Dental Hospital. Inclusion criteria for CUs were as follows: (1) no complaint of subjective memory problems; (2) normal cognitive function, defined by a Mini-Mental State Examination (MMSE) score above the 16th percentile for age- and education-matched norms; (3) Clinical Dementia Rating (CDR) Scale 0; (4) no history of neurologic or psychiatric disorders.

Mouth clinical examinations

Plaque samples were obtained from all participants at the Department of Periodontics of Pusan National University Dental Hospital, Yangsan, Korea. All AD patients and CUs had moderate-to-advanced periodontal disease, including probing depth (PD) > 5 mm, clinical attachment loss (CAL) > 3 mm, and radiographic evidence of extensive bone loss. Full-mouth clinical examinations including PD, CAL, gingival index (GI) and plaque index (PI) were carried out by one practitioner.

The assessment for periodontal disease includes number and type of teeth present, PD in millimeters and location of the gingival margin in relation to the cementoenamel junction at six locations/tooth (mesiobuccal, midbuccal, distobuccal, mesiolingual, midlingual, distolingual) using a UNC-15 manual probe (HuFriedy, Chicago, IL, USA). PD was measured as the linear distance in millimeters from the gingival margin to the base of the periodontal pocket. CAL was computed by adding the PD to the distance from the free gingival margin to the cementoenamel junction. GI involves a scale from 0 to 3 for the buccal, lingual, mesial and distal surfaces that is scored as follows: 0 indicates healthy gums; 1 indicates slight color changes, light edema and no presence of bleeding on probing; 2 indicates edema with slight redness and bleeding on probing; and 3 indicates severe edema, redness, the presence of ulceration and a tendency for spontaneous bleeding (23). PI was evaluated by staining tooth surface with 2.0% Erytrosin highlighter and scores of 0 to 5 were recorded for the buccal and lingual surfaces. In this scoring system, 0 indicated no visible plaque, and 5 indicated that more than 2/3 of the tooth surface was covered in plaque (24).

Neuropsychological tests

All AD patients underwent neuropsychological tests using a standardized neuropsychological battery called the Seoul Neuropsychological Screening Battery (SNSB) when they were initially diagnosed with AD (25). At the time that plaque samples were collected, all participants including AD and CU performed Korean version of the Mini-Mental State Examination (K-MMSE), and CDR.

Plaque Sample Collection

Buccal, supragingival and subgingival plaque samples were collected with the full-mouth periodontal examination. Buccal swab sample was obtained from mucosa of both cheeks with a sterile microbrush and placed in a separate sterile 1.5 ml microcentrifuge tube. All supragingival plaque samples were collected from each participant from mesiobuccal sites of molars. Subgingival plaque samples were

obtained at the time of periodontal surgery. Samples from all subjects were collected and stored at −80◦C for subsequent processing.

Extraction of Genomic DNA and Next Generation Sequencing

Total DNA was extracted from the buccal, supragingival and subgingival plaque using a Gram positive DNA purification kit (Lucigen, Biosearch Technology, Novato, CA) following the manufacturer's instructions. The final concentration was measured with a NanoDrop ND-1000 spectrophotometer (Thermo Fisher Scientific, USA) and stored at − 80◦C until use. PCR amplification of the 16S ribosomal RNA gene V3-V4 region was performed. Purified amplicons were pooled in equimolar and paired-end sequenced with HiSeq (Illumina, San Diago, CA, USA). Raw fastq files were demultiplexed and processed using tools available in QIIME2 (version 2019.7). Reads were demultiplexed with q2-demux and quality filtered and dereplicated with q2-dada2. Representative sequence sets for each dada2 sequence variant were used for taxonomy classification with naïve Bayes machine-learning classifier (26).

Bioinformatic Analysis, Statistical Analysis, and Visualization

The mean clinical parameters were then compared via a paired Student's t-test. After rarefying the OTU table, three metrics were calculated for the evaluation of alpha diversity: Chao 1 estimates the species abundance; and the diversity of the sample microbiota was estimated by the Shannon and Simpson index. The Mann–Whitney U test was used to compare significant differences of the alpha diversity indexes between the different groups ($p < 0.05$). To evaluate the similarity of microbial community structure among all samples, a principal coordinates analysis (PCoA) was performed on the species level. Analysis of similarities was calculated to compare based on the Bray-Curtis distance which was calculated by R. The non-parametric permutation multivariate analysis of variance (PERMANOVA) test, implemented in the Adonis function of the R/vegan package was used to identify group differences with 1,000 permutations. (27). Taxa that have an abundance significantly different among groups were identified by linear discriminant analysis effect size (LEfSe) (28) with default settings. To visualize the internal interactions and further measurement of the microbial community, strong, prosperous, and resilient communities challenge (SparCC) (29) was used to calculate the Spearman correlation coefficient with corresponding P value between each two species. The network was then visualized by R/igraph package with the nodes denoting the species, and connections representing the existence of correlation.

Results

Patient characterization

Total of 29 subjects were recruited in the study, including 14 CU participants and 15 AD patients. The demographic and clinical parameters of the participants are detailed in Table 1. Although age was significantly higher in the AD group, there were no significant difference between the CU and AD groups in

periodontitis-related parameters including PD, CAL, GI and PI, and remaining number of teeth. Education and MMSE were significantly lower in the AD group.

Diversity and abundance of microbiota

We used 16S rRNA V3-V4 targeted sequencing to characterize the microbiota composition. The alpha diversity of the microbiota was estimated by Chao1, Shannon and Simpson index (Fig. 1A). In buccal and supragingival samples, there was no significant difference between CU and AD groups in all the tests. In subgingival samples, AD showed significantly higher index in Choa1 and Shannon. To analyze the distribution of microbiota, the beta diversity analysis with PCoA was conducted based on the species abundances. A PERMANOVA test between two groups showed that the association of microbiome composition was significantly different in the subgingival samples while we could not find the significance for the tests in the other two samples. Furthermore, PCoA revealed that the individual samples were not clustered well in the buccal and supragingival samples compared to the subgingival samples (Fig. 1B).

At the phyla level, in buccal and supragingival samples of the CU and AD groups, the five most abundant of the phyla were Firmicutes, Proteobacteria, Bacteroidetes, Actinobacteria, and Fusobacteria, which represented more than 96% of the total sequences. In samples of subgingival plaque, the aforementioned five phyla constituted the majority of the sequences, while the abundance of Spirochaetes were increased in both groups. The abundance of Bacteroidetes (8.6% vs 16.5%) and Fusobacteria (8.5% vs 15.5%) were increased in subgingival samples of AD patients, whereas the abundance of Actinobacteria (23.8% vs 9.5%) were increased in subgingival samples of CU people (Fig 2A).

At the genus level, among the 333 genera detected, the most abundant genera in the buccal samples of both groups were *Streptococcus, Haemophilus, Rothia, Gemella* and *Neisseria*. These constituted more than 75% of the total sequences. In supragingival plaque samples of both groups, *Streptococcus, Rothia, Haemophilus, Lautropia* and *Fusobacterium* represented more than 50% of the total sequences. In subgingival plaque samples of both groups, *Streptococcus, Fusobacterium, Porphyromonas, Rothia,* and *Haemophilus* represented more than 45% of the total sequences. Depending on sampling sites, there was a shift of bacterial abundance. The average abundance of *Fusobacterium* and *Porphyromonas* was 2.35%, 1,38% at buccal mucosa, 4.23%, 3.1% at supragingival plaque, and 9.49%, 4.68% at subgingival plaque, respectively. Moreover, *Fusobacterium* and *Porphyromonas* were also more abundant in AD group (7.15%, 11.81%) compared to CU group (4.11%, 5.25%) in subgingival plaque (Fig. 2B).

Species taxa comparison

To discriminate the bacterial species abundance between two groups, AD and CU groups, the LEfSe algorithm has been used at the species level. In the buccal samples, *Haemophilus parahaemolyticus, Porphyromonas gingivalis, Treponema denticola,* and *Prevotella enoeca* were significantly more

abundant in CU group compared to AD group. The abundance of *Prevotella baroninae, Erythrobacter litoralis, Phocaeicola abscessus,* and *Leptotrichia hofstadii* were significantly higher in the AD group (Fig. 3A). In the supragingival samples, *Porphyromonas gingivalis, Filifactor alocis, Aggregatibacter aphrophilus* and *Eikenella corrodens* were significantly more abundant in CU group compared to AD group. The abundance of *Prevotella veroralis, P. oulorum, P. salivae,* and *Capnocytophaga sputigenae* were significantly higher in the AD group (Fig. 3B). In the subgingival samples, *Actinomyces oris, Actinomyces dentalis, Rothia aeria, Filifactor alocis, Aggregatibacter aphrophilus* and *Eikenella corrodens* were significantly more abundant in CU group compared to AD group. The abundance of various *Prevotella* species including *P. micans, P. saccharolytica, P. maculosa, P. nigrescens, P. denticola, P. oralis,* and *P. buccae* were significantly higher in the AD group (Fig. 3C).

Network Analysis

Using abundance profiles for the microbiome communities in the sample, we are able to create microbial interaction networks to provide information on potential interaction patterns among microbes. We generated bacterial interaction network maps using differentially abundant taxa determined by LEfSe analysis in the subgingival samples. The network analysis results showed that the bacterial interaction patterns between two groups were distinctive in terms of the network formations. In the CU group, *Alloprevotella tannerae, Selenomonsa sputigena, Oribacterium sinus, P. oralis, Atopobium rimae*, and *Dialister invisus* were highly interconnected (Fig. 4A). On the other hand, in the AD group, *Lautrophia mirabilis, Chryseobacterium, Cardiobacterium hominis, Corynebacterium durum,* and *R. denticariosa* formed highly interconnected network and the hub species, *Schwartzia succinivorans* interacted with *Oribacterium sinus* and *P. nigrescens* (Fig. 4B).

Discussion

In the elderly, the prevalence of CP is more than 50% and the prevalence of AD is around 5% (30–32). Poor oral hygiene are associated with AD (12) and CP exposure is associated with increase in the risk of developing AD (10). Emerging evidence suggested a link between human microbiota and neurodegenerative diseases. Usage of antibiotics has been reported to improve AD (33, 34), which support the hypothesis of interaction between microbiome and AD development. Herpes viruses are thought to be transferred to brain via peripheral nerve (35). It has been hypothesized that periodontal pathogens may enter the brain via similar route. The identification of oral Treponema in the trigeminal ganglia also supports the neural route (22). Oral bacteria are found in the brain tissue and cerebrospinal fluid of autopsy cases with AD (18, 19, 22). Serum antibodies to periodontal pathogens are a risk factor for Alzheimer's disease (36). Nevertheless, only few studies focused on the relation between the oral microbiome and AD (20). In this study, we characterized the oral microbiome of AD with CP compared with cognitive healthy CP patients with compatible oral condition. Clinical parameters were evaluated and oral samples were collected from buccal, supragingival and subgingival plaque for microbiome analysis.

When the alpha diversity of the microbiota was estimated, there was no significant difference between CU and AD groups in buccal and supragingival samples, suggesting that overall microbiome richness and evenness were similar between two groups. Meanwhile, in subgingival samples, AD showed significantly higher index in Choa1 and Shannon, suggesting a subgingival microbiome profile was more complex in the AD group. In studies that compared AD to healthy control, the alpha diversity in AD patients was significantly lower in both saliva and gut (16, 37). However, alpha diversity of subgingival plaque was higher in CP than in periodontally healthy controls, which may result from the formation and accumulation of dental plaque biofilms indicating periodontal disease status (38, 39). That is, higher diversity of microbiome in periodontitis is related to pathogenic state. When the beta diversity of the microbiota was compared by PCoA, the results showed that the individual samples in each group were clustered together in the subgingival samples, which suggest that the beta diversity of subgingival microbiome is different between two groups.

Our study showed similar overall composition in subgingival samples between the CU and AD groups. The five most abundant of the 9 phyla were Firmicutes, Proteobacteria, Bacteroidetes, Actinobacteria, and Fusobacteria. These findings are in line with previous studies that showed subgingival microbiota of patients with periodontitis (38). However, the AD group had larger proportion of Bacteroidetes and Fusobacteria than the CU group in the present study (Fig. 2A). Composition of oral microbiome in subgingiva can be a role in AD pathophysiology related to oral disease.

In our study, at the genus level, the most abundant bacteria in buccal and supragingival plaques were *Streptococcus, Haemophilus, and Rothia*. In subgingival plaques, *Streptococcus, Fusobacterium, Porphyromonas, Rothia*, and *Haemophilus* were frequent. Depending on sampling sites, there was a shift of bacterial abundance. The average abundance of *Fusobacterium* and *Porphyromonas* were 2.35%, 1,38% at buccal mucosa, 4.23%, 3.1% at supragingival plaque, and 9.49%, 4.68% at subgingival plaque, respectively. In subgingival space, the environmental properties such as temperature (40) and the oxygen tension (41) change compared to the oral cavity. In AD, the abundance of *Fusobacterium* and *Porphyromonas* was higher compared to CU, suggesting that subgingival ecosystem may differ between CU and AD despite overall clinical scores were similar.

In the comparison of microbiota between CU and AD according to sampling sites, we found that the abundant microbiota in the AD group was not in accordance with well-known periodontal pathogens. Generally, gram-negative *Porphyromonas gingivalis, Treponema denticola, Tannerella forsythia, Prevotella intermedia, Fusobacterium nucleatum, Filifactor alocis* and *Eikenella corrodens* have been considered as major pathogens of periodontal disease (42–44). In buccal samples, *P. gingivalis* and *T. denticola* were more abundant in CU patients and the abundances of *Prevotella baroninae* and *Leptotrichia hofstadii* were significantly higher in the AD group. In the supragingival samples, *P. gingivalis, F. alocis*, and *E. corrodens* were more abundant in the CU group and the abundances of *Prevotella veroralis, P. oulorum, P. salivae*, and *Capnocytophaga sputigenae* were significantly higher in AD group. Although not many species were significantly different between the two groups, the CU group showed higher abundance of well-known periodontal pathogens in buccal and supragingival sites,

whereas the AD group did not. Through these results, we suggest that ecological conditions of oral mucosa are not same in CU and AD. In terms of subgingival samples, *F. alocis* and *E. corrodens* were among the significant taxa more abundant in the CU group. Previous studies that studied a role of periodontal pathogens modulating brain inflammation focused on well-known pathogens including *P. gingivalis* (45, 46). Interestingly, we found various *Prevotella* species were significantly more abundant in AD. *Prevotella* species are anaerobic Gram-negative bacteria of the Bacteroidetes phylum, which also includes the clinically important genera *Bacteroides* and *Porphyromonas* (47). *Prevotella* species have been found to be prevalent commensal colonizers at mucosal sites; being the predominant genus in the respiratory system (48, 49) and a central constituent in gut bacterial enterotypes (50, 51), as well as present in saliva and oral cavity (52, 53). Only a few *Prevotella* strains have been reported to give rise to opportunistic endogenous infections, including chronic infections, abscesses and anaerobic pneumonia (54, 55). However, emerging studies have linked increased *Prevotella* abundance and specific strains to inflammatory disorders, suggesting that at least some strains of *Prevotella* exhibit pathologic properties. Serum antibody levels specific for *F. nucleatum* and *Prevotella intermedia* are correlated with cognitive deficits (36). In a mouse study, *Prevotella nigrescens* induced periodontal disease and the infected mice exhibited accelerated onset and severity of experimental arthritis compared with control mice (56). Abundance of *Prevotella* in subgingival plaque of AD can implicate the possibility that specific bacteria in CP are associated with AD.

Finally, the interactions among the oral microbiota community were analyzed to provide information on potential interaction patterns of microbes. We created networks using significant abundant taxa determined by LEfSe analysis in the subgingival samples. We found distinctive network complexity in each CU group and AD group in terms of the network formation. The more co-abundance correlations in the networks indicate strong symbiosis in the community. Oral cavity is colonized with an intricate community of indigenous microorganisms, where the microbiota with their internal interactions are shaping a reticular system to maintain the composition (57–59). Accordingly, the distinctive network structure in microbial community is likely to induce characteristic immune response to mediate inflammatory responses necessary for host defense. However, these network maps represent patterns, and not direct interactions. Many of the observed interactions may be due to microbes sharing a similar ecological niche. Further studies are warranted to determine the inflammatory responses induced depending on distinctive microbiome structure, and to reveal the causal relationship of dysbiosis of oral microbiota, related metabolic activity and AD progression.

Limitations

Although the study results are very clear, there are limitations to our study. Firstly, the findings from the present study with a small sample will need to be further validated in future well-designed larger scale. Although power calculation is not feasible to this study, adequate number of participants are needed to allow stratification of participants into different groups of sex, clinical stage, education, or life style which may influence the result. Secondly, to determine whether the characteristic oral microbiome in AD is the cause or result of the disease, well designed longitudinal studies with adequate sample size are needed

to elucidate the role of oral pathogens in AD development. Further, although clinical scores related with periodontitis were thoroughly measured in this study, clinical measurements can be difficult for elderly participants to complete. More simplified methods to measure oral parameters should be applied for further study.

Conclusion

In conclusion, we compared the oral microbiome structure in CP patients with AD and CU patients. The overall clinical parameters and structure of buccal and supragingival oral microbiome was similar between two groups. However, the richness and diversity of subgingival microbiome of AD patients were significantly increased compared to CU patients. Also, the composition of subgingival microbiome was different between CU and AD groups. Thus, our results provide novel understanding on the role of the oral microbiome in AD patients. By improving oral hygiene and regular monitoring, the composition of oral microorganisms can be modulated, which may provide an important and feasible way to reduce the risk of dementia in the elderly population. Further well-designed studies with an adequate sample size are required to confirm our results and draw more definitive conclusions.

Abbreviations

AD: Alzheimer's disease; CP: Chronic periodontitis; CU: Cognitively unimpaired; MMSE: Mini-Mental State Examination; CDR: Clinical Dementia Rating; PD: probing depth; CAL: clinical attachment loss; GI: gingival index; PI: plaque index; SNSB: Seoul Neuropsychological Screening Battery; K-MMSE: Korean version of the Mini-Mental State Examination; PCoA: principal coordinates analysis; PERMANOVA: permutation multivariate analysis of variance; LEfSe: linear discriminant analysis effect size; SparCC: strong, prosperous, and resilient communities challenge

Declarations

Ethics approval and consent to participate

The procedure was performed following current guidelines and regulations, and the experimental protocol was approved by the Institutional Review Board at each hospital (PNUDH-2017-023, PNUYH-05-2017-140). Written informed consent was obtained from all participants before the study.

Consent for publication

Not applicable.

Availability of data and materials

The raw sequencing data have been deposited at NCBI GenBank under BioProject ID PRJNA625189 (BioSample SAMN14594851 - SAMN14594936). Please check the data using the below private reviewer

link, https://dataview.ncbi.nlm.nih.gov/object/PRJNA625189?reviewer=qmbf1r25iigbp7mt9h3ngvqjqk

Competing interests

None of the contributors to this study have any conflict of interest to declare.

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Author's contributions

HSN, NYJ, and JC outlined the research project. HSN, NYJ, and JIC designed the experiment. NYJ, HJK, JYL, JIC, and SYK coordinated the data collection. SYK performed the experiment. SC, HSN, JHL and YHK performed data analysis. HSN and NYJ drafted the original manuscript, and JHL and JC revised the original draft and contributed relevant suggestions to the final manuscript. All authors revised and approved the final paper draft.

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Author's information

Department of Oral Microbiology, School of Dentistry, Pusan National University; Oral Genomics Research Center, Pusan National University, Yangsan, 50612, South Korea

Hee Sam Na, Si Yeong Kim & Jin Chung

Department of Neurology, Pusan National University Yangsan Hospital, Pusan National University School of Medicine and Research Institute for Convergence of Biomedical Science and Technology, Yangsan, 50612, South Korea

Na-Yeon Jung

Interdisplinary Program of Genomic Science, Pusan National University, Yangsan, 50612, South Korea

Suji Choi & Yun Hak Kim

Department of Periodontology, School of Dentistry, Pusan National University; Dental Research Institute, School of Dentistry, Pusan National University, Yangsan, 50612, South Korea

Hyun-Joo Kim, Ju Youn Lee & Jeom-Il Choi

Department of Maxillofacial Biomedical Engineering, School of Dentistry, Kyung Hee University; Department of Life and Nanopharmaceutical Sciences, Kyung Hee University; Kyung Hee Medical Science Research Institute, Kyung Hee University, Seoul, 20447, South Korea

Jae-Hyung Lee

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Figures

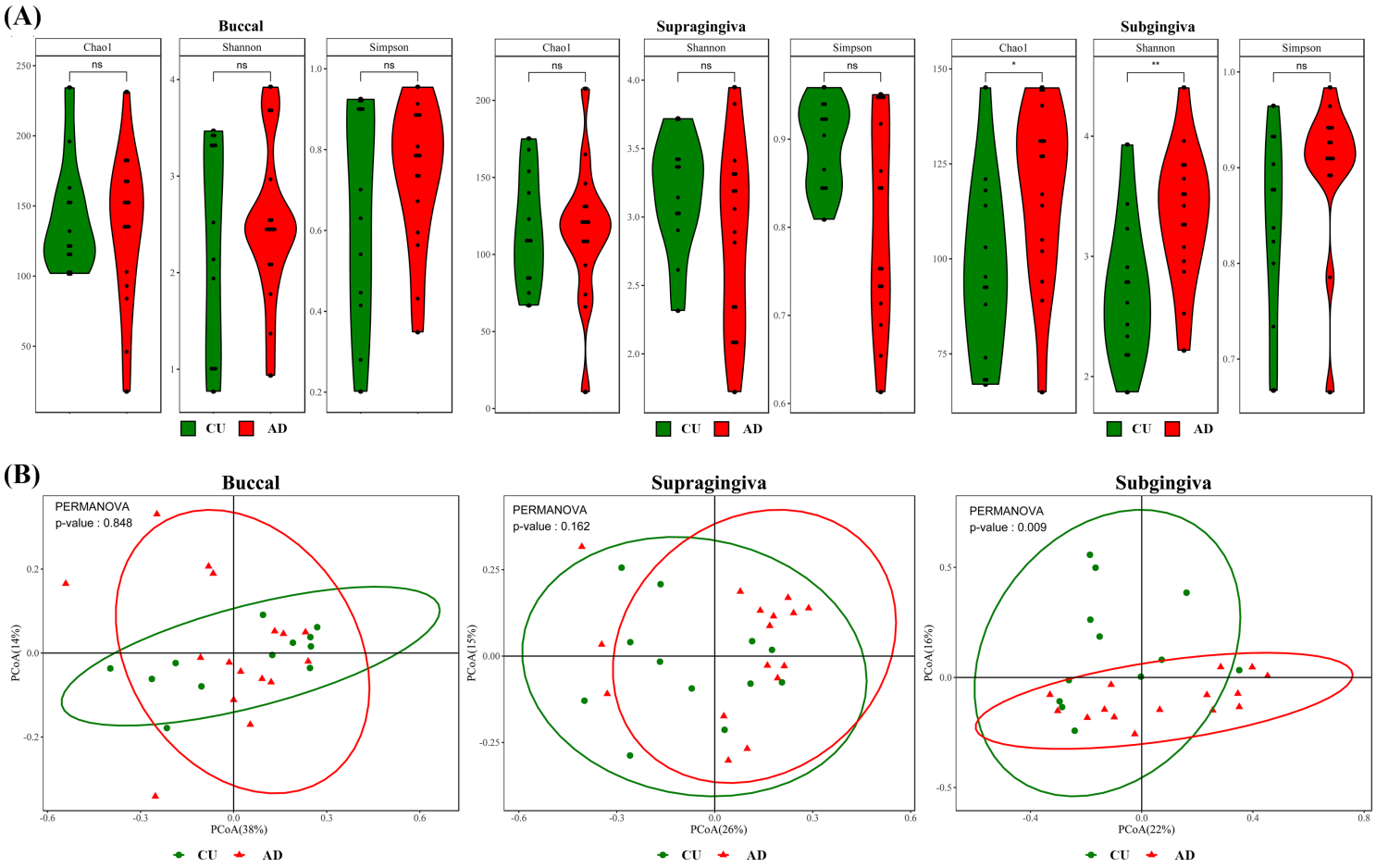

Figure 1

Bacterial community comparisons between CU and AD. (A) Alpha diversity (Chao1, Shannon and Simpson indices) of buccal samples, supragingival and subgingival plaque. (B) Beta diversity of buccal samples, supragingival and subgingival plaque. The non-parametric PERMANOVA test has been used to identify the group differences between CU and AD. Principal coordinates analysis (PCoA) were performed based on the species abundances. CU, cognitive unimpaired; AD, Alzheimer's disease. * p<0.05, ** p<0.01

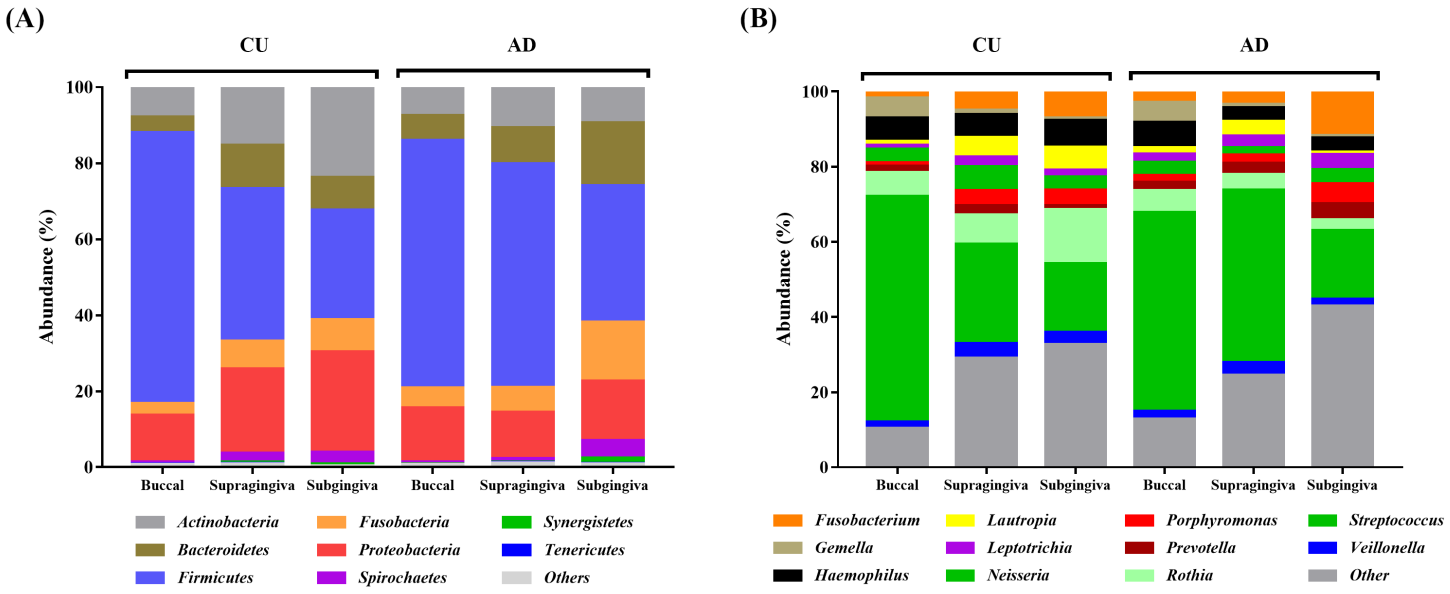

Figure 2

Relative abundance of bacterial composition of buccal samples, supragingival and subgingival plaques in CU and AD. (A) Phylum level (B) Genus level. CU, cognitive unimpaired; AD, Alzheimer's disease.

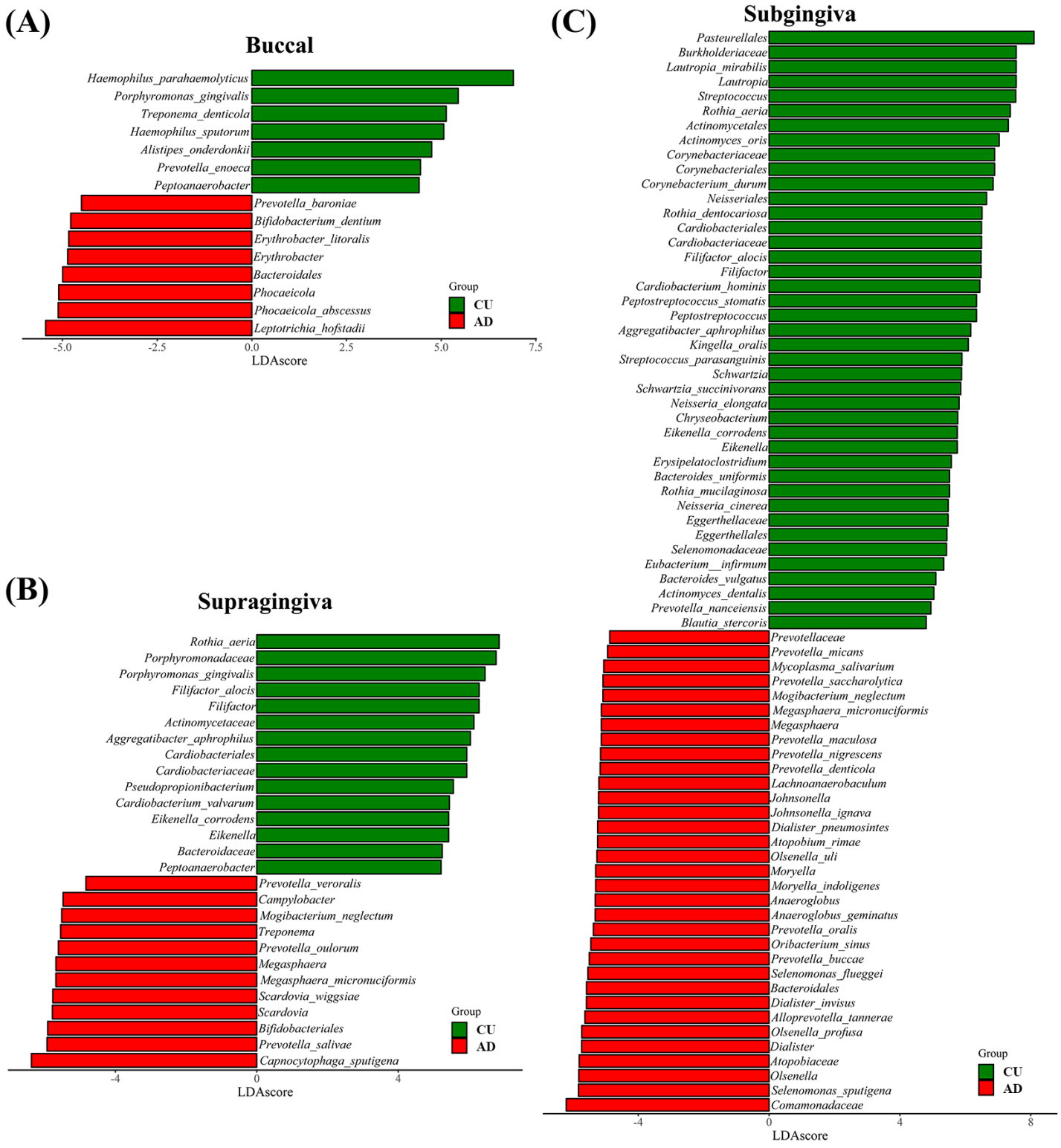

Figure 3

Comparisons of microbiota between CU and AD that presented significantly different contents in buccal samples (A), supragingival (B) and subgingival (C) plaque. The analysis has been performed using linear discriminant analysis effect size (LefSe) method. CU, cognitive unimpaired; AD, Alzheimer's disease.

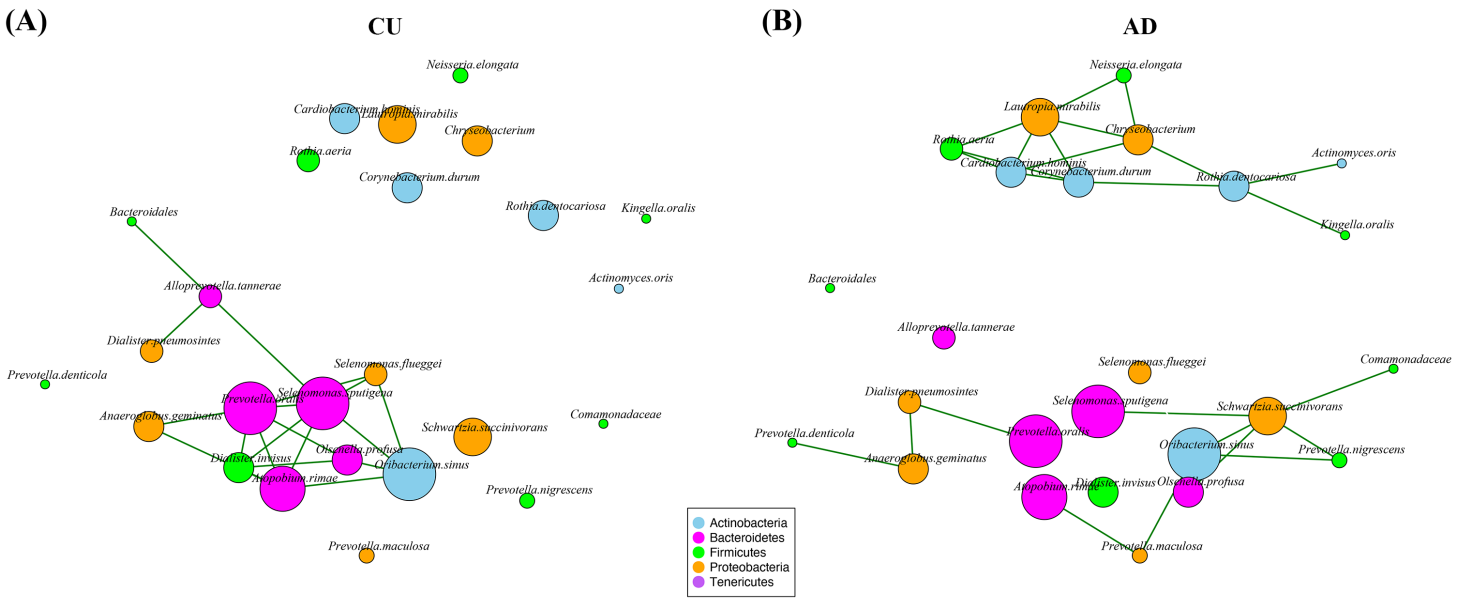

Figure 4

Subgingival microbiome network analysis of CU (A) and AD (B). Each bubble represents a species whose color varied with phylum according to the legend. A connection between two bubbles indicated existence of the correlation between the two corresponding species. CU, cognitive unimpaired; AD, Alzheimer's disease.