

Oral microbiome perturbations link periodontal health to cognitive ageing in a large community cohort



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Summary

Background Emerging evidence implicates the oral-brain axis in neurodegeneration, yet large community-based studies remain limited. This study aimed to examine associations between periodontal health, oral microbiome, and cognitive performance, and to explore potential biological pathways underlying these relationships.

Methods We conducted a cross-sectional analysis of 1157 participants from the community-based Taizhou Imaging Study, all of whom underwent comprehensive periodontal examinations, salivary microbiome profiling, and cognitive assessments. Periodontal health and microbiome features were treated as exposures, and cognitive performance as the outcome. Associations between periodontal indices and cognitive scores were assessed using beta regression models adjusted for relevant confounders. Cognition-related microbial features were identified using Multivariate Associations with Linear Models (MaAsLin3), followed by mediation analyses to explore potential pathways linking periodontal health to cognitive function.

Findings Five clinical periodontal indices were found to be inversely associated with cognitive performance. Ten microbial genera (e.g., *Haemophilus*), 21 functional pathways (e.g., FoxO signalling), and two co-abundance modules, including a *Treponema* module, were significantly related to cognitive function. Mediation analysis suggested that 11 features, including nitrate-reducing taxa and a *Treponema*-driven inflammatory module, may partially mediate the relationship between periodontal health and cognition.

Interpretation These community-based findings reveal microbiome-mediated links along the oral-brain axis and highlight periodontal health and oral microbial homeostasis as potential targets for early prevention of cognitive decline.

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Keywords: Periodontitis; Cognitive impairment; Oral microbiome; Oral-brain axis; Mediation

Research in context

Evidence before this study

Periodontal inflammation and oral microbial dysbiosis may induce systemic oxidative stress and neuroinflammatory responses along the oral-brain axis, contributing to cognitive decline, yet community-based evidence remains limited.

Added value of this study

By integrating comprehensive periodontal examinations with deep salivary microbiome profiling in 1157 community-dwelling older adults, our findings provide a comprehensive salivary microbiome atlas spanning periodontitis and cognitive status, revealing concordant β -diversity stratification across groups. We found nitrate-reducing microbial features (including *Haemophilus*) aligned with better cognitive performance, whereas inflammatory

metabolic traits (including a *Treponema*-enriched inflammatory module) were associated with impaired cognitive performance. Further mediation analyses identify 11 microbial features that significantly mediate the relationship between periodontal health and cognition.

Implications of all the available evidence

Our study offers a population-based perspective on the oral-brain axis, showing that periodontal health is significantly associated with cognitive function. Inflammatory mediators and nitrate-reducing bacteria may serve as key mechanistic links underlying this relationship. Collectively, these findings advance our understanding of oral-brain pathways and highlight potential translational targets for interventions focused on improving oral hygiene to support brain health.

Introduction

Cognitive impairment and dementia represent major global health challenges, with China particularly affected due to its rapidly ageing population.^{1,2} Although genetic predisposition contributes to disease susceptibility, accumulating evidence emphasises the pivotal role of lifestyle and other modifiable factors in shaping cognitive trajectories,³ highlighting the importance of identifying actionable targets for prevention. Among these, oral health—historically overlooked in the context of neurodegeneration—has emerged as a potentially influential and tractable determinant, offering promising opportunities for early intervention and preservation of cognitive function.^{4,5} This consideration is especially urgent in China, where oral health status is generally poor,^{6,7} amplifying the potential impact of oral-related interventions.

Epidemiological evidence increasingly supports an association between oral health and cognitive outcomes, with periodontal status representing a core element of this relationship.⁸ Periodontitis may initiate a systemic inflammatory cascade through the release of pro-inflammatory mediators, adversely affecting multiple systemic conditions^{9,10} and potentially contributing to neuroinflammation and amyloid deposition in the brain.^{11,12} Individuals with poorer oral hygiene, tooth loss, or more severe periodontitis consistently exhibit lower cognitive performance and elevated risk of cognitive decline.¹³ Conversely, structured oral care, including oral hygiene instruction and supportive periodontal therapy, has been shown to enhance periodontal status and reduce inflammation.^{14,15} Notably, active periodontal treatment such as prophylaxis and

subgingival scaling has been associated with favourable effects on dementia-related brain atrophy.¹⁶ In parallel, less frequent oral care and suboptimal oral hygiene behaviours, including reduced toothbrushing frequency, are linked to poorer cognitive outcomes.⁵ Although causal inference remains limited, these findings collectively underscore periodontal health as a potentially modifiable determinant of cognitive trajectories,⁸ prompting growing interest in elucidating the biological mechanisms linking oral and brain health and identifying potential interventional targets.

Among the proposed mechanisms linking periodontal health to cognitive function, microbial dysregulation has emerged as a central pathway.^{17–19} Accumulating molecular, immunological, and epidemiological evidence has increasingly linked oral dysbiosis to cognitive impairment.^{17,20} Several oral pathogens and toxic proteases have been detected in brain tissue from patients with Alzheimer's disease (AD) such as *Porphyromonas gingivalis* and *gingipains*.²¹ Serum antibody levels against specific oral bacteria are altered in individuals with cognitive impairment. Elevated antibodies against *Fusobacterium nucleatum* and *Prevotella intermedia* have been observed prior to the clinical diagnosis of mild cognitive impairment (MCI) and AD,²² while higher anti-*Eubacterium nodatum* IgG levels are associated with a lower risk of AD.²³ Furthermore, multiple studies have reported shifts in oral microbial diversity, specific taxa, and metabolite profiles across different cognitive states.^{24,25} However, most existing evidence is derived from case-control or small-sample studies, and few have integrated periodontal indices, oral microbiome profiling, and

cognitive assessment within a large, community-based population.^{25–27}

Building on accumulating evidence linking oral health to cognitive impairment and its emerging potential as a modifiable target to prevent cognitive decline, we conducted a comprehensive investigation integrating clinical periodontal examinations, cognitive assessments, and salivary microbiome profiling in a large community-based cohort, the Taizhou Imaging Study. This study aimed to: (1) identify periodontal indices associated with cognitive performance; (2) systematically characterise associations between the oral microbiome, periodontal indices, and cognitive function; and (3) explore potential microbiome-mediated pathways linking periodontal health to cognition through mediation analysis, thereby providing mechanistic insights into the oral-brain axis (Fig. 1).

Methods

Study design and participants

This cross-sectional analysis was nested within the Taizhou Imaging Study (TIS), an ongoing community-based cohort study investigating risk factors and multi-omics biomarkers for age-related diseases in older Chinese adults. The TIS Phase I enrolled 904 Han Chinese participants between 2013 and 2018, as previously described.²⁸ Major exclusion criteria included a history of dementia, stroke, cancer, and severe psychiatric or organ disorders. During the second follow-up visit in 2020, oral health assessments were incorporated into the study protocol (response rate 75.7%). In 2021, Phase II expanded the cohort by recruiting 634 additional participants aged 50–70 years from a different community within the same region (response rate 76.4%), following the same inclusion and exclusion

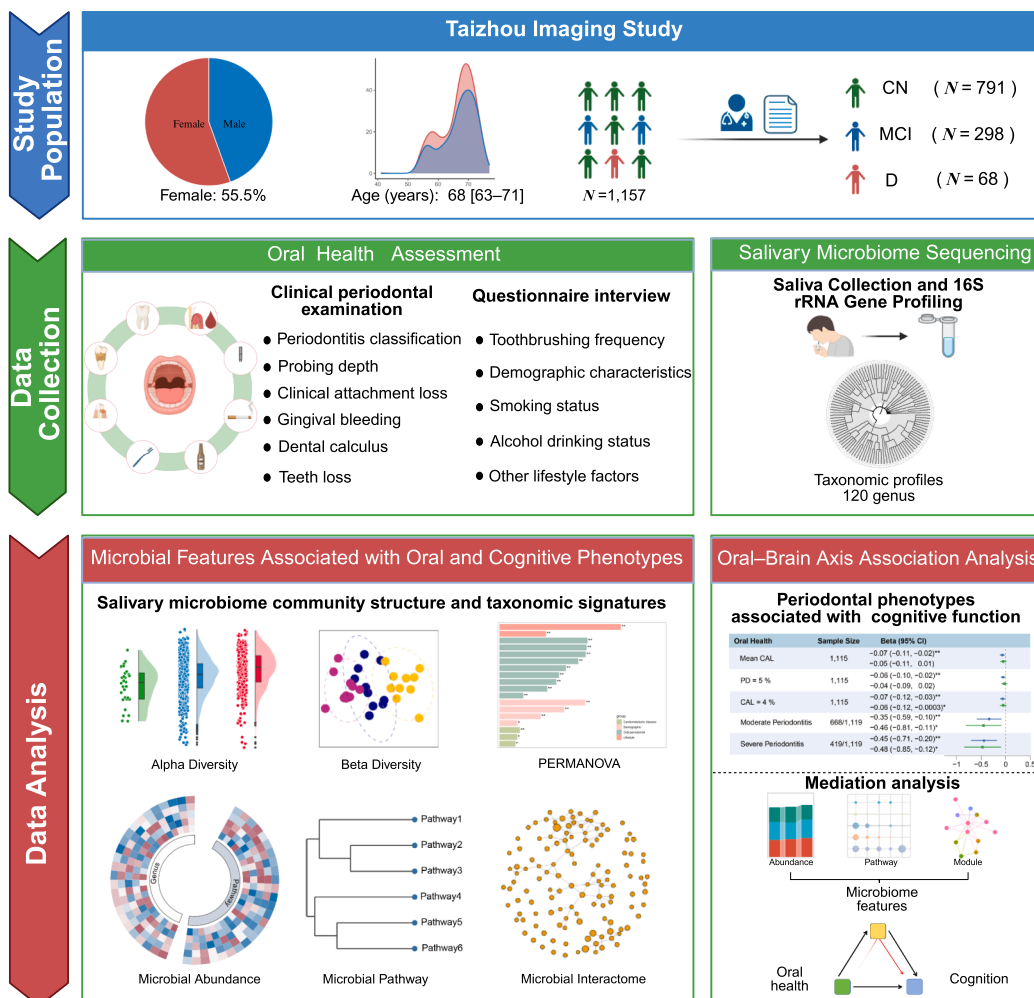


Fig. 1: Study design and analytical workflow. Note: Part of the figure was created with ©BioRender (biorender.com), in accordance with BioRender's terms and conditions.

criteria and assessment procedures as Phase I, with oral health assessments conducted from baseline.

For the present study, we combined data from Phase I's second follow-up (2020; $N = 709$) and Phase II's baseline (2021; $N = 634$), both of which included oral health examinations, saliva samples, and cognitive assessments (Supplementary Figure S1A). After excluding participants with insufficient saliva for 16S sequencing ($N = 161$) or incomplete cognitive data ($N = 25$), a total of 1157 participants were included in the analysis (Supplementary Figure S1B). Details on sample size estimation calculations are provided in the Supplementary Material.

Cognitive function assessment

Cognitive function was assessed in quiet testing rooms using a standardised neuropsychological test battery,²⁸ all of which have been validated in Chinese populations.^{29,30} The battery covered multiple domains: global cognition was evaluated with the Mini-Mental State Examination (MMSE) and the Beijing version of the Montreal Cognitive Assessment (MoCA); memory was assessed using the Auditory Verbal Learning Test (AVLT); attention and executive function with the Trail Making Test (TMT) and a Go/No-Go task; language using the Animal Fluency Test; and visuospatial ability with the Clock Drawing Test.

Participants were classified as cognitively normal (CN), MCI, or dementia, according to the Diagnostic and Statistical Manual of Mental Disorders (DSM-IV, 4th edition) criteria and Petersen's criteria.^{31,32} Diagnostic adjudication was performed by a consensus panel of neurologists and neuropsychologists integrating medical history/records, neurological examination findings, and neuropsychological test results. Among the 1157 included participants, 791 were classified as CN, 298 as MCI, and 68 as dementia; cognitive impairment (CI) was defined as MCI or dementia.

Periodontal assessment and diagnosis of periodontitis

All participants underwent a full-mouth periodontal assessment according to the WHO criteria,³³ including the number of missing teeth, calculus index, gingival bleeding index, probing depth, and clinical attachment loss, as described previously.³⁴ For gingival bleeding index, probing depth, and clinical attachment loss, participants were randomly assigned to receive half-mouth examinations (quadrant I and III or quadrant II and IV), a validated alternative to full-mouth assessments in population-based studies.³⁵ Gingival bleeding index and calculus index were evaluated on the labial (buccal) and lingual (palatal) surfaces of each tooth, while probing depth and clinical attachment loss were measured at six sites per tooth: mesio-buccal, mid-buccal, disto-buccal, mesio-lingual, mid-lingual, and disto-lingual.

Periodontitis was defined using the Centres for Disease Control and Prevention/American Academy of Periodontology criteria,³⁶ which show high consistency with European Federation of Periodontology and American Academy of Periodontology criteria.^{37,38} In light of the half-mouth protocol, we applied a reduced Centres for Disease Control and Prevention/American Academy of Periodontology definition. Mild periodontitis was defined as ≥ 1 interproximal site with clinical attachment loss ≥ 3 mm and ≥ 1 interproximal site with probing depth ≥ 4 mm. Moderate periodontitis was defined as ≥ 1 interproximal site with clinical attachment loss ≥ 4 mm or probing depth ≥ 5 mm. Severe periodontitis was defined as ≥ 1 interproximal site (not on the same tooth) with clinical attachment loss ≥ 6 mm and ≥ 1 interproximal site with probing depth ≥ 5 mm.^{39,40} To further capture disease complexity, we also calculated the proportion of sites with probing depth ≥ 5 mm and clinical attachment loss ≥ 4 mm. Specifically, given the distinct microbial profiles between edentulous individuals and those with periodontitis,⁴¹ coupled with the small sample size (Table 1), edentulous participants were treated as a separate group and therefore excluded from downstream analyses.

Saliva sample collection and 16S rRNA gene sequencing

Saliva samples were collected from participants in a fasting state, after abstaining from drinking, smoking, or chewing gum for at least 30 min. Following a sterile water rinse, approximately 2 mL of unstimulated whole saliva was collected by drooling into tubes prefilled with stabilisation buffer. Tubes were promptly capped and gently inverted to ensure homogenisation. All samples were collected prior to periodontal examination, immediately frozen on-site at -20°C , and transferred within 4 h for long-term storage at -80°C until DNA extraction.

Total DNA was extracted from 100 μL of saliva using the OMEGA Soil DNA Kit (M5635-02; Omega Bio-Tek, Norcross, GA, USA) according to the manufacturer's instructions. DNA concentration and integrity were evaluated using a NanoDrop NC2000 spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA) and agarose gel electrophoresis.

The V3–V4 region of the bacterial 16S rRNA gene was amplified using primers 338F (5'-ACTCCTACGG-GAGGCAGCA-3') and 806R (5'-GGACTACHVGGGT-WTCTAAT-3'), with sample-specific 7-bp barcodes to enable multiplexed sequencing. Each 25 μL PCR reaction mixture contained the following components: 5 μL of $5 \times$ reaction buffer, 0.25 μL of FastPfu DNA polymerase (5 U/ μL), 2 μL of dNTPs (2.5 mM), 1 μL of each forward and reverse primer (10 μM), 1 μL of template DNA, and 14.75 μL of ddH₂O. The thermal cycling conditions were as follows: initial denaturation at 98°C for 5 min; 25 cycles of denaturation at 98°C for 30 s, annealing at 53°C for 30 s, and extension at 72°C for

Characteristics	Total (N = 1157)	CN (N = 791)	MCI (N = 298)	Dementia (N = 68)	P value
Ethnicity					–
Han Chinese (%)	1157 (100.0)	791 (100.0)	298 (100.0)	68 (100.0)	
Age (years)	68.0 [63.0, 71.0]	67.0 [61.0, 70.0]	69.0 [66.0, 72.0]	71.0 [68.0, 73.0]	<0.001
Sex					<0.001
Male (%)	515 (44.5)	378 (47.8)	128 (43.0)	9 (13.2)	
Female (%)	642 (55.5)	413 (52.2)	170 (57.0)	7 (26.5)	
BMI (kg/m ²)	23.4 [21.3, 25.7]	23.6 [21.6, 25.9]	23.3 [21.2, 25.4]	22.5 [20.5, 25.3]	0.050
Education (years) ^a	6.0 [1.0, 8.0]	6.0 [3.0, 9.0]	6.0 [0.0, 8.0]	0.0 [0.0, 1.5]	<0.001
Smoker ^a	398 (34.7)	285 (36.4)	103 (34.9)	10 (14.9)	0.002
Alcohol drinker ^a	380 (33.2)	288 (36.7)	81 (27.5)	11 (16.4)	<0.001
APOE ε4 carrier ^a	185 (16.9)	123 (16.5)	49 (17.3)	13 (20.0)	0.759
MMSE	25.0 [21.0, 28.0]	26.0 [24.0, 28.0]	22.0 [18.0, 26.0]	12.0 [9.8, 14.3]	<0.001
MoCA	17.5 [13.0, 21.3]	19.0 [15.0, 22.5]	14.0 [9.0, 18.0]	5.0 [3.0, 7.3]	<0.001
Daily toothbrushing frequency ^a					0.004
0–1 time	616 (53)	417 (52.7)	156 (52.3)	43 (63.2)	
2–3 time	332 (29)	246 (31.1)	79 (26.5)	7 (26.5)	
Severity of periodontitis ^a					0.422
No/Mild	32 (2.8)	26 (3.3)	5 (1.7)	1 (1.5)	
Moderate	669 (57.8)	464 (58.7)	170 (57.0)	35 (51.5)	
Severe	419 (36.2)	278 (35.1)	112 (37.6)	29 (42.6)	
Edentulous	13 (1.1)	8 (1.9)	3 (1)	2 (2.9)	
MT ^a	1.0 [0.0, 4.0]	1.0 [0.0, 4.0]	2.0 [1.0, 5.0]	2.0 [1.0, 5.5]	<0.001
GBI sites (%) ^a	0.4 [0.2, 0.7]	0.5 [0.3, 0.7]	0.4 [0.2, 0.6]	0.4 [0.2, 0.7]	0.909
Calculus sites (%) ^a	0.7 [0.5, 0.9]	0.7 [0.5, 0.9]	0.7 [0.6, 0.9]	0.7 [0.5, 0.9]	0.079
Mean CAL ^a (mm)	3.0 [2.5, 3.6]	3.0 [2.5, 3.5]	3.1 [2.6, 3.7]	3.0 [2.6, 3.8]	0.187
Mean PD ^a (mm)	1.8 [1.5, 2.2]	1.8 [1.5, 2.2]	1.8 [1.5, 2.2]	1.7 [1.4, 2.2]	0.381
Hypertension	554 (47.9)	374 (47.3)	141 (47.3)	39 (57.4)	0.273
Diabetes	158 (13.7)	106 (13.4)	40 (13.4)	12 (17.6)	0.614
Hyperlipidaemia	620 (53.6)	430 (54.4)	150 (50.3)	40 (58.8)	0.332

Note: Data are presented as frequency (%) for categorical variables and as median [25th, 75th percentile] for continuous variables. P values indicate overall differences across the three cognitive groups. **Abbreviations:** CN, cognitively normal; MCI, mild cognitive impairment; BMI, body mass index; MMSE, Mini-Mental State Examination; MoCA, Montreal Cognitive Assessment; MT, missing teeth; GBI sites (%), percentage of sites with gingival bleeding on probing; CAL, clinical attachment loss; PD, periodontal probing depth; Calculus sites (%), percentage of sites with dental calculus. ^aMissing data: education (0.3%), smoker (1.0%), alcohol drinker (1.0%), APOE ε4 carrier (5.4%), daily toothbrushing frequency (18%), periodontitis severity (3.2%), MT (2.0%), GBI sites (3.6%), Calculus sites (3.7%), mean CAL (3.5%), and mean PD (3.5%).

Table 1: Characteristics of study participants stratified by cognitive groups.

45 s; followed by a final extension at 72 °C for 5 min. The PCR products were purified using VAHTS™ DNA Clean Beads (Vazyme, Nanjing, China). Final libraries were quantified with the Quant-iT PicoGreen dsDNA Assay Kit (Invitrogen, Carlsbad, CA, USA) and the purity of libraries was measured on an Agilent 2100 Bioanalyzer. Following quantification, amplicons from each sample were pooled in equimolar amounts to generate a composite library, which was subjected to paired-end sequencing (2 × 250 bp) on an Illumina NovaSeq platform with the NovaSeq 6000 SP Reagent Kit (500 cycles). All sequencing was performed by Shanghai Personal Biotechnology Co., Ltd. (Shanghai, China).

Microbiome data processing and analysis

Sequencing data processing. Raw paired-end reads were processed in QIIME2 (version 2022.11).⁴² After demultiplexing (with the *demux* plugin) and primer

removal (with *cutadapt*), sequences were quality-filtered, trimmed, denoised, merged and inferred as amplicon sequence variants (ASVs) using DADA2.⁴³ To reduce noise, low-prevalence ASVs (present in <1% of samples) were removed, yielding a final set of 891 ASVs.

Taxonomic and functional profiling. Taxonomy was assigned using a Naive Bayes classifier trained on the SILVA 138 reference database.⁴⁴ We identified ASVs assigned to 13 phyla, 20 classes, 43 orders, 62 families, and 120 genera across all samples. Functional profiles were inferred with Tax4Fun2,⁴⁵ generating predictions for 6570 Kyoto Encyclopedia of Genes and Genomes (KEGG) orthologs (KOs) and 311 level-3 KEGG pathways (Supplementary Figure S2). Taxonomic features and pathways with relative abundance of <0.01% in >90% of samples were excluded,^{46–48} resulting in 108 genera and 201 pathways retained for further analysis.

Diversity analyses. Alpha Diversity was quantified using the Shannon, Simpson, Pielou, and Chao1 indices.^{49,50} Between-group differences in alpha diversity were tested with the Kruskal–Wallis test, followed by Dunn’s post hoc tests for pairwise comparisons, with statistical significance defined as Benjamini–Hochberg false discovery rate (FDR)-adjusted $P < 0.05$. Beta diversity was assessed using Bray–Curtis dissimilarities calculated from ASV-level relative abundances to capture differences in community composition. Group-level differences and the proportion of variance explained by host factors were assessed using permutational multivariate analysis of variance (PERMANOVA; adonis2, vegan v2.6-10 in R; 999 permutations), with FDR-adjusted $P < 0.05$ considered significant. Beta diversity patterns were visualised with principal coordinates analysis (PCoA) based on Bray–Curtis distances.

Differential abundance analysis. Differentially abundant taxa between disease-status groups were identified using linear discriminant analysis effect size (LEfSe) as implemented in the “microeco” R package, across all available taxonomic levels. Taxa with Kruskal–Wallis $P < 0.05$ and linear discriminant analysis (LDA) score > 2.0 were considered significant.

Salivary microbial signature identification. Mixed-effects models were then fitted with MaAsLin3 (v3.0) to examine associations between microbial features and periodontal health as well as cognitive function.⁵¹ Associations for microbial taxa were assessed using both abundance-based and prevalence-based models, whereas all other feature types were analysed using abundance-based models only.

Microbial interactome analysis. Species-level co-abundance modules were inferred using Sparse Correlation Network Investigation for Compositional Data (SCNIC) with default parameters and a correlation threshold of $r > 0.30$.⁵² Associations between module abundances and cognitive as well as periodontal measures were assessed using MaAsLin3 (v3.0) under a mixed-effects framework.

Covariates

Demographic and lifestyle information, including age, sex, education level, daily toothbrushing frequency, current smoking and alcohol consumption status, as well as medical and medication history, were collected via a structured questionnaire administered by trained technicians, which were audio-recorded, reviewed on-site, and verified within the secure cohort database to ensure accuracy and reliability.²⁸ Sex was self-reported at enrolment (female or male), with no restrictions applied during study design or recruitment, and was included as a covariate in the analyses. Participants underwent physical examinations, blood pressure measurements, and fasting blood collection. Body mass index (BMI) was calculated as weight in kilograms divided by height in metres squared (kg/m^2). Serum

was analysed for total cholesterol, triglycerides, and other clinical biomarkers using an automated biochemical analyser.⁵³

Hypertension was defined as systolic/diastolic blood pressure $\geq 140/90$ mmHg, a prior diagnosis, or current use of antihypertensive medication. Diabetes was defined as fasting plasma glucose ≥ 7.0 mmol/L, a prior diagnosis, or use of glucose-lowering agents. Hyperlipidaemia was defined as total cholesterol ≥ 5.2 mmol/L, triglycerides ≥ 1.7 mmol/L, a history of hyperlipidaemia, or current use of lipid-lowering medication.

Genome-wide genotyping was performed using the Axiom Precision Medicine Research Array (PMRA) on de-identified genomic DNA extracted from whole blood. *APOE* genotype was determined from the rs7412 and rs429358 variants,⁵⁴ and participants carrying at least one $\epsilon 4$ allele were classified as *APOE* $\epsilon 4$ carriers.

Statistical analyses

Descriptive statistics summarised demographic, lifestyle, and clinical characteristics across cognitive groups. Continuous variables were compared using Student’s *t*-test or the Wilcoxon rank-sum test, and categorical variables were compared using Pearson’s χ^2 test or Fisher’s exact test, as appropriate.

Multivariable beta regression models were fitted to assess the associations between periodontal health (exposure) and cognitive scores (outcomes) using the R package “betareg”,^{55,56} with model diagnostics detailed in the [Supplementary Material](#). Covariates were selected a priori for their potential relevance to oral health and cognitive function.^{12,25} Two successive adjustment models were applied: Model 1 adjusted for age (continuous), sex (male/female), BMI (continuous), education level (literacy, primary school, junior middle school, or high school and above), and *APOE* $\epsilon 4$ carrier status (carrier/non-carrier); Model 2 additionally adjusted for current smoking (yes/no), current alcohol consumption (yes/no), and daily toothbrushing frequency (low: 0–1 times/day; high: 2–3 times/day). Statistical significance was set at $P < 0.05$.

Associations between microbial features (exposure) and periodontal or cognitive indices (outcomes) were evaluated with MaAsLin3 (v3.0) mixed-effects models, adjusting for prespecified covariates and including a random intercept for enrolment batch. For microbial–periodontal associations, Model 1 adjusted for age, sex, BMI, and education level, incorporating a random intercept for sample enrolment batch. Model 2 additionally included current smoking, alcohol consumption, and daily toothbrushing frequency. For microbial–cognition associations, Model 1 included the same covariates and random effect, whereas Model 2 additionally adjusted for current smoking, alcohol consumption, and *APOE* $\epsilon 4$ carrier status. Significance was determined using a false discovery rate (FDR)-adjusted $q < 0.1$.^{51,57}

In the primary association analysis, participants with missing covariate data were excluded (complete-case analysis). To evaluate the robustness of the results, two sensitivity analyses were performed: (1) repeating the analyses with multiple imputation (R package “MICE”) to handle missing covariates,⁵⁸ and (2) additionally adjusting models for clinical comorbidities, including diabetes, dyslipidaemia, and hypertension.

Mediation analyses were performed to investigate whether oral microbiome features mediated the relationship between periodontal health and cognitive function. The associations between periodontal health and cognitive function, as well as the oral microbiome features, were initially evaluated in the primary association analyses (Model 2, $P < 0.05$). Pairs that exhibited significant associations in the per-feature tests were retained for mediation analysis. Mediation effects were estimated using linear mixed-effects models implemented via the R package “mediation”, with adjustment for age, sex, BMI, education level, current smoking, current alcohol consumption, daily toothbrushing frequency, and *APOE* $\epsilon 4$ carrier status, and including a random intercept for enrolment batch. Microbial features with an average causal mediation effect (ACME; $P < 0.05$) were considered statistically significant.

Consent statement

The Taizhou Imaging Study (TIS) was approved by the Ethics Committee of the Fudan University Taizhou Institute of Health Sciences (institutional review board approval number B017). All participants provided written informed consent authorising the use of their data and biospecimens for research purposes only.

Role of funders

Funders of the study had no role in study design, data collection, data analyses, interpretation, or writing of report.

Results

Participant characteristics across cognitive groups

Participant demographics, lifestyle factors, medical history, and cognitive profiles are summarised in [Table 1](#). BMI, apolipoprotein E (*APOE*) $\epsilon 4$ carrier status, and vascular comorbidities were comparable across groups. Compared with CN participants, those with MCI or dementia were older, less educated (Kruskal–Wallis test, all $P < 0.05$); less often male, and had lower proportions of smokers and drinkers (Pearson’s χ^2 test, all $P < 0.05$). Cognitive scores, as measured by the Mini-Mental State Examination (MMSE) and the Beijing Montreal Cognitive Assessment (MoCA), were correspondingly lower in the MCI and dementia groups (Kruskal–Wallis test, both $P < 0.05$). Notably, individuals with CI reported less frequent daily toothbrushing (Pearson’s χ^2 test, $P < 0.05$) and exhibited

more missing teeth (Kruskal–Wallis test, $P < 0.05$), consistent with a potential relationship between oral health and cognition.

Associations between periodontal status and cognitive performance

We evaluated the relationship between periodontal health and cognitive performance using beta regression models for MMSE and MoCA scores, adjusting for demographic, genetic, and lifestyle covariates ([Fig. 2A](#); [Supplementary Table S1](#)). Among nine periodontal health measures, five were significantly associated with cognition: the percentage of sites with dental calculus, mean clinical attachment loss (CAL), the proportion of sites with probing depth (PD) ≥ 5 mm, the proportion of sites with CAL ≥ 4 mm, and periodontitis severity (Beta regression, all $P < 0.05$). These findings remained robust in sensitivity analyses ([Supplementary Table S2](#)).

Distinct oral microbiome profiles across periodontitis and cognitive groups

The salivary microbiome was dominated by Bacteroidota (34.6%), Bacillota (27.0%), Pseudomonadota (19.0%), and Actinomycetota (7.9%) at the phylum level, with *Prevotella* (21.3%), *Streptococcus* (15.8%), *Neisseria* (12.4%), and *Actinomyces* (5.6%) as the most abundant genera ([Fig. 2B](#)).

Global analyses revealed that periodontitis severity was associated with increased α -diversity (Shannon, Simpson, Pielou’s evenness, and Chao1 indices) and distinct β -diversity differences between no/mild and moderate/severe periodontitis ([Fig. 3A](#) and [B](#)). In contrast, α -diversity did not differ across cognitive status ([Supplementary Figure S3A](#)), whereas β -diversity showed separation between CN and CI individuals (MCI or dementia), with no detectable distinction between MCI and dementia ([Fig. 3B](#)).

Permutational multivariable analysis of variance (PERMANOVA) indicated that lifestyle and periodontal factors, particularly smoking, mean PD, and periodontitis severity, accounted for the largest proportion of microbial variance (3.53%), exceeding demographic, cognitive, and other disease-related factors ([Fig. 3C](#)). Mantel tests confirmed significant correlations between predominant periodontal indicators and the relative abundance of major genera ([Fig. 3D](#)). These results indicate strong associations between periodontal status and oral microbial variation, which may relate to cognitive performance.

Gradients of oral microbial composition across periodontitis and cognitive strata

Linear discriminant analysis effect size (LEfSe) analyses identified 186 taxa differing by periodontitis severity, and 70 taxa differing across cognitive groups (linear discriminant analysis (LDA) score > 2 , $P < 0.05$ by Kruskal–Wallis test; [Supplementary Figure S3B](#),

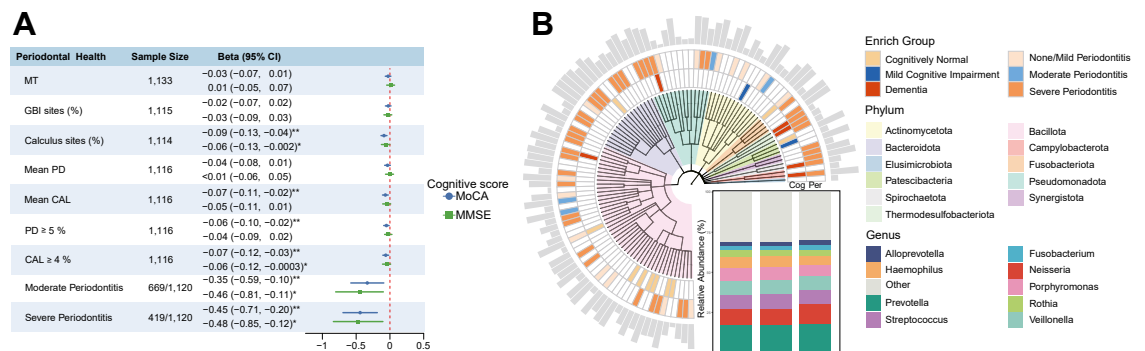


Fig. 2: Associations between periodontal health and cognition (A) and oral microbiome taxonomic features (B) in the Taizhou Imaging Study. Note: (A) β coefficients were estimated from beta regression models. * $P < 0.05$; ** $P < 0.01$. **(B)** Phylogenetic tree of the salivary microbiome, highlighting genera significantly enriched across cognitive and periodontitis status groups (LEfSe, LDA >2.0 , $P < 0.05$ by Kruskal-Wallis test). The outer ring indicates each genus's prevalence among participants. Companion bar plots, aligned to the phylogeny, display the ten most abundant genera within each cognitive category. **Abbreviations:** MT, missing teeth; GBI sites (%), percentage of sites with gingival bleeding on probing; Calculus sites (%), percentage of sites with calculus; Mean PD, Mean periodontal probing depth; Mean CAL, Mean clinical attachment loss; PD $\geq 5\%$, percentage of sites with probing depth ≥ 5 mm; CAL $\geq 4\%$, percentage of sites with clinical attachment loss ≥ 4 mm; MMSE, Mini-Mental State Examination; MoCA, Montreal Cognitive Assessment; LEfSe, linear discriminant analysis effect size; LDA, linear discriminant analysis.

Supplementary Figure S4; Supplementary Table S3). Specifically, across periodontitis strata, the no/mild periodontitis group exhibited enrichment of genera including *Haemophilus*, *Rothia*, and *Schaalia* (15 genera in total) and species such as *Haemophilus parainfluenzae*, *Rothia mucilaginosa*, and *Streptococcus sanguinis* (20 species in total). The moderate periodontitis group showed enrichment of genera including *Streptococcus*, *Granulicatella*, and *Slackia* (four genera in total) and species such as *Porphyromonas pasteri* and *Schaalia odontolytica* (nine species in total). The severe periodontitis group was characterised by enrichment of genera including *Treponema*, *Neisseria*, and *Fusobacterium* (42 genera in total) and species such as *P. intermedia*, *P. gingivalis*, and *F. nucleatum* (49 species in total) (Supplementary Figure S4, Supplementary Table S3).

Correspondingly, across cognitive strata, the CN group showed enrichment of genera including *Porphyromonas*, TM7x, and *Pseudoleptotrichia* (10 genera in total) and species such as *H. parainfluenzae*, *R. mucilaginosa*, and *Veillonella rogosae* (17 species in total). The MCI group exhibited enrichment of genera including TM7a and *Bifidobacterium*, and species such as *Streptococcus caballi*. In contrast, the dementia group was characterised by enrichment of genera including *Campylobacter*, *Sphaerochaeta*, and *Anaeroglobus* (six genera in total) and species such as *Veillonella parvula*, *F. nucleatum*, and *Prevotella denticola* (16 species in total) (Supplementary Figure S3B; Supplementary Table S3).

Oral microbial signatures associated with periodontal and cognitive status

Microbiome Multivariable Associations with Linear Models (MaAsLin3) analyses identified 63 genera

associated with periodontal measures (Fig. 4A; Supplementary Table S4). Among these genera, *Treponema*, *Solobacterium*, and *Dialister* were positively associated with increasing periodontal inflammation, whereas *Haemophilus*, *Rothia*, and TM7x showed inverse associations. Notably, a general pattern emerged in which more abundant genera were more frequently inversely associated with periodontal inflammation, while less abundant genera tended to show positive associations (Fig. 4A). Functional profiling revealed 70 pathways significantly linked to periodontal health (Fig. 4A, Supplementary Table S5). Pathways related to bacterial colonisation, motility, and immune activation (e.g., flagellar assembly and NOD-like receptor signalling) were enriched, alongside depletion of systemic homeostasis pathways (e.g., glutamatergic and GABAergic synapses).

Ten genera were significantly associated with cognitive function (Fig. 4A, Supplementary Table S6). *Rothia*, *Haemophilus*, and TM7x showed positive associations with cognitive performance, while *Abiotrophia* was significantly negatively associated with MoCA scores. Functional analyses identified 21 cognitive-related pathways (Fig. 4A), encompassing lipid and energy metabolism, ageing, immune response system, and infectious diseases-related pathways (Supplementary Table S7). For instance, ribosome biogenesis in eukaryotes (ko03008) and FoxO signalling pathway (ko04068) were positively associated with cognitive performance, whereas epithelial cell signalling in *Helicobacter pylori* infection (ko05120) and NOD-like receptor signalling pathway (ko04621) were negatively associated.

Sensitivity analyses identified consistent microbial features associated with periodontal incidence

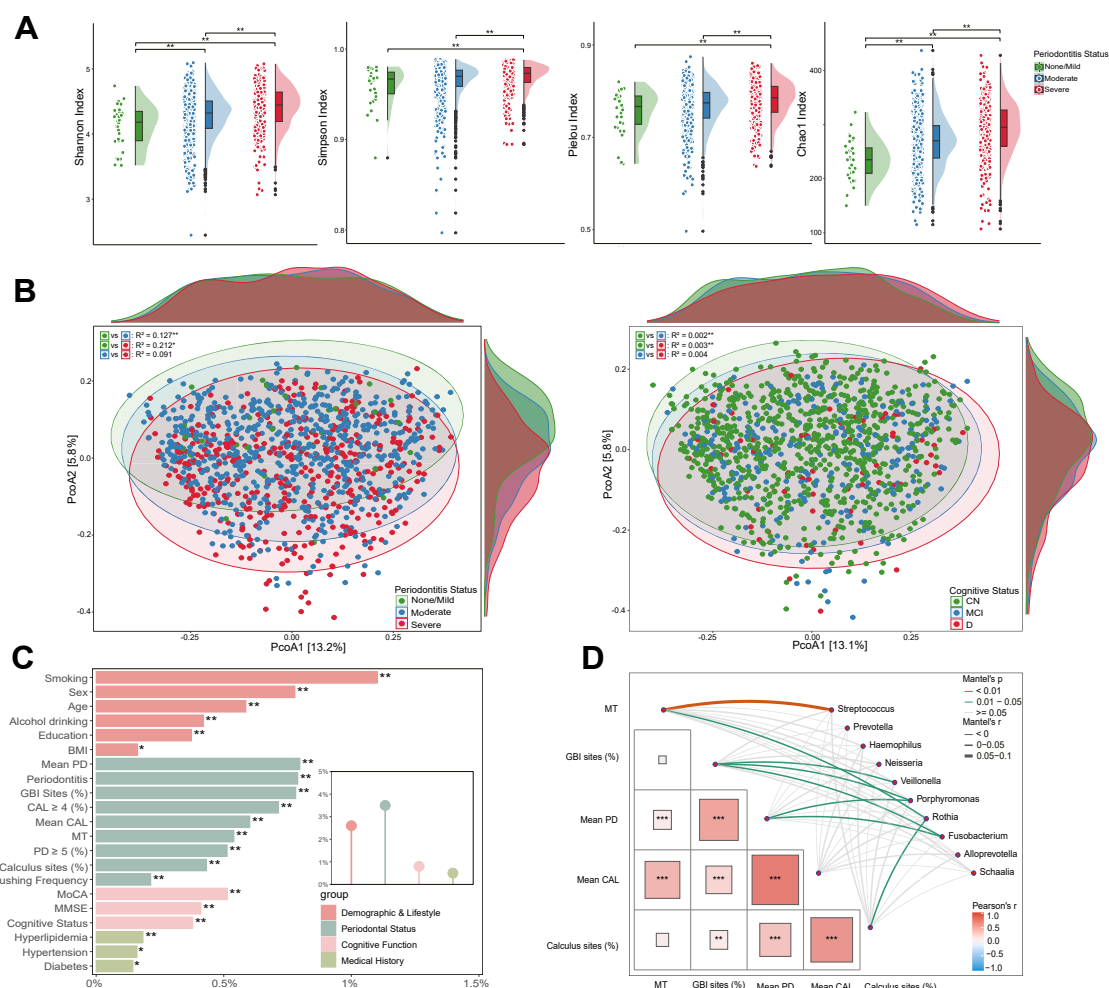


Fig. 3: Oral microbiome diversity and community associations with periodontitis and cognitive status in the Taizhou Imaging Study.

Note: (A) Raincloud plots of α -diversity indices (Shannon, Simpson, Pielou, and Chao1) across periodontitis groups. Following a significant Kruskal-Wallis test, pairwise differences were assessed using Wilcoxon rank-sum tests with Benjamini-Hochberg false discovery rate correction, and significant comparisons are indicated. *FDR- $P < 0.05$; **FDR- $P < 0.01$. (B) Principal Coordinates Analysis (PCoA) of microbial community structure based on Bray-Curtis dissimilarities. PERMANOVA results (Adonis R^2 and P value) are shown. *FDR- $P < 0.05$; **FDR- $P < 0.01$. (C) Variance in salivary microbiome composition explained by periodontal, cognitive, and other host factors. Bar plots display the proportion of variation for each variable; inset summarises variance across four phenotype categories. (D) Correlations between periodontal indices and the ten most abundant oral genera. Lower-left matrix: pairwise Pearson correlations (square area is proportional to $|r|$; red-blue colour indicates direction). Right network: links each index to genera (edge thickness is proportional to Mantel's r ; edge colour denotes Mantel P). **Abbreviations:** CN, cognitively normal; MCI, mild cognitive impairment; D, dementia; MT, missing teeth; GBI sites (%), percentage of sites with gingival bleeding on probing; Mean PD, Mean periodontal probing depth; Mean CAL, Mean clinical attachment loss; Calculus sites (%), percentage of sites with calculus; PD $\geq 5\%$, percentage of sites with probing depth ≥ 5 mm; CAL $\geq 4\%$, percentage of sites with clinical attachment loss ≥ 4 mm; MMSE, Mini-Mental State Examination; MoCA, Montreal Cognitive Assessment; PERMANOVA, permutational multivariate analysis of variance.

measures and cognitive performance (Supplementary Tables S8–15), confirming the robustness of the primary findings.

Co-abundance microbial modules linking periodontal and cognitive profiles

Seventeen species-level co-abundance modules were constructed using Sparse Correlation Network Investigation

for Compositional Data (SCNIC) (Fig. 4B; Supplementary Table S16). Nine modules were associated with periodontal health indicators, and two with cognitive function (MaAsLin3, Fig. 4C; Supplementary Table S17, Supplementary Table S18). Modules enriched in early colonisers and nitrate-reducing commensals (e.g., M3: *Actinomyces graevenitzi*, *S. odontolytica*; M8: *Neisseria subflava*, *H. parainfluenzae*) were associated with better

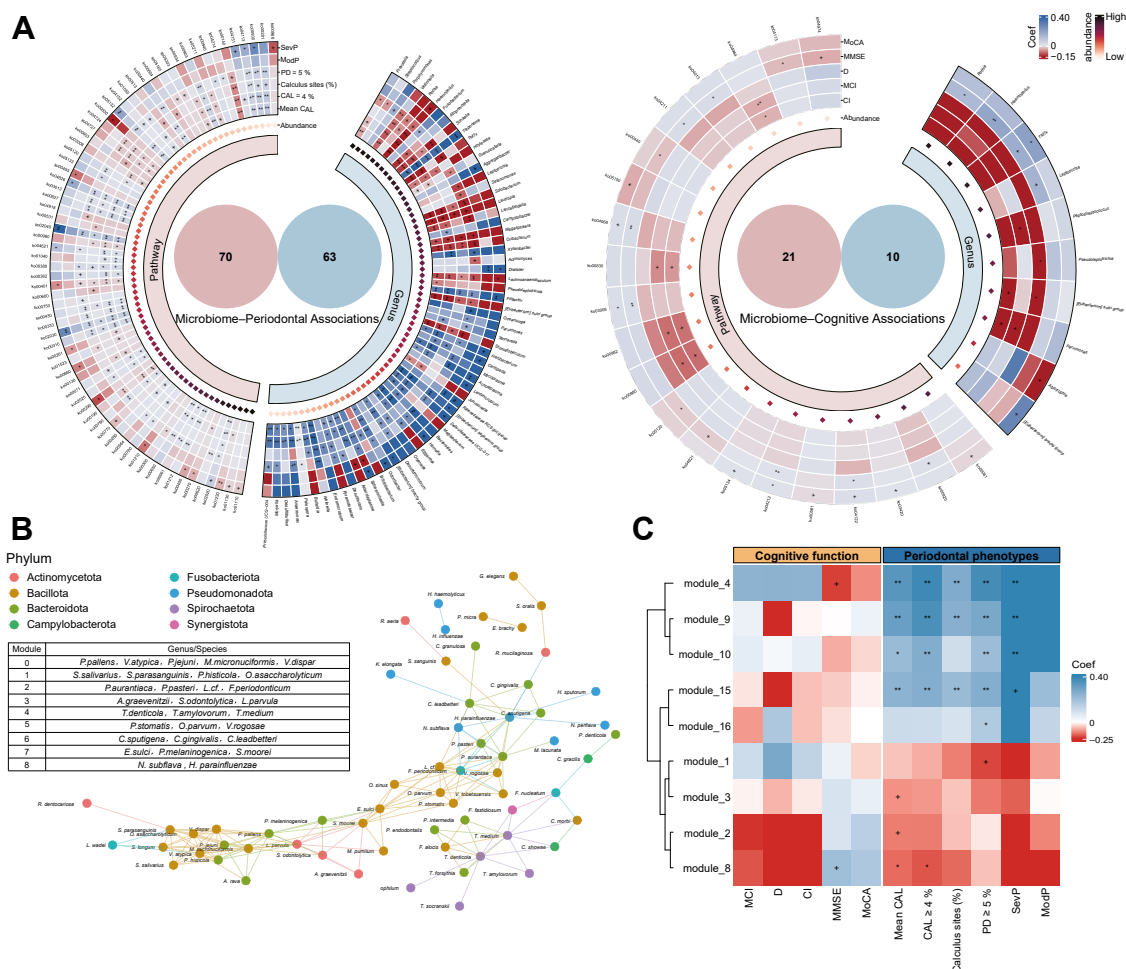


Fig. 4: Integrative Analysis of Oral Microbiome Features Links Periodontal Health with Cognitive Function. Note: (A) Circos plot of genera and pathways significantly associated with periodontal and cognitive indicators. Inner circles denote the number of significant features; the outer ring shows correlation heatmaps, with rows as clinical metrics and columns as microbial features ranked by abundance. Tile colour indicates correlation strength; adjacent dots indicate features with significant associations identified using MaAsLin3 (Multivariable Association with Linear Models 3), with false discovery rate adjustment ($q < 0.10$). For genera significant in both abundance- and prevalence-based models, the abundance model is shown by priority. *FDR- $P < 0.1$; *FDR- $P < 0.05$; **FDR- $P < 0.01$. **(B)** SparCC co-occurrence network of 165 microbial species, with node colour representing phylum. SCNIC identified 17 modules, of which 8 contained ≥ 3 members. **(C)** Heatmap of modules significantly associated with periodontal health and cognition (MaAsLin3), with false discovery rate adjustment ($q < 0.10$). Blue and pink denote positive and negative correlations, respectively. *FDR- $P < 0.1$; *FDR- $P < 0.05$; **FDR- $P < 0.01$. **Abbreviations:** SevP, severe periodontitis; ModP, moderate periodontitis; PD $\geq 5\%$, percentage of sites with a probing depth ≥ 5 mm; Calculus sites (%), percentage of sites with dental calculus; CAL $\geq 4\%$, percentage of sites with clinical attachment loss ≥ 4 mm; Mean CAL, Mean clinical attachment loss; MMSE, Mini-Mental State Examination; MoCA, Montreal Cognitive Assessment; D, dementia; MCI, mild cognitive impairment; CI, cognitive impairment; SCNIC, Sparse Correlation Network Investigation for Compositional Data.

periodontal and cognitive profiles, whereas modules dominated by inflammation-associated periodontopathogenic anaerobes (M4: *Treponema denticola*, *T. amylovorum*, *T. medium*; M9: *Tannerella forsythia*, *Filifactor alocis*) were negatively associated. These coordinated microbial communities may contribute to the observed links between periodontal status and cognition. Results from two sensitivity analyses were consistent with primary findings; notably, both Module 4 and

Module 8 remained significantly associated with MMSE scores (Supplementary Table S19–22).

Mediation of periodontal-cognition associations by the oral microbiome

In silico mediation analyses examined whether oral microbiome features partially mediated the association between periodontal health and cognition. Eleven microbial features, including key genera, functional

pathways, and co-abundance network modules, formed 24 significant mediation paths (Average Causal Mediation Effects [ACME]- $P < 0.05$; Fig. 5; Supplementary Table S23–25). Among these, TM7x and ribosome biogenesis in eukaryotes each mediated four paths, while *Haemophilus*, *Treponema*, M8, and M4 modules emerged as significant mediators.

Discussion

In this large community-based cross-sectional study of 1157 adults, key periodontal indicators were inversely associated with cognitive performance. We further identified multiple microbial genera, functional pathways, and co-abundance modules related to cognition, and mediation analysis suggested that oral microbiome features may partly link periodontal inflammation to cognitive outcomes. To our knowledge, this large-scale community-based cohort uniquely integrates comprehensive periodontal assessment, cognitive evaluation, and oral microbiome profiling to

investigate potential mediation pathways between oral health and cognition.

Consistent with prior population-based analyses, including analyses of the NHANES database⁵⁹ and a 5-year cohort study linking severe periodontitis to MCI in older adults,⁶⁰ our current and previous findings further support an association between periodontal deterioration and cognitive impairment.³⁴ Potential mechanistic pathways include systemic inflammation, microbial translocation, platelet aggregation, and immune modulation, all of which may influence neuronal function.^{26,61} These findings, while compelling, remain observational and should be interpreted as associations rather than causal effects.

Periodontal deterioration and cognitive impairment were associated with pronounced remodelling of the oral microbiome, both compositionally and functionally. Progressive periodontitis coincided with increased microbial richness and evenness and enrichment of classic pathogens, including *P. gingivalis*, *T. forsythia*, and *T. denticola*, as well as other anaerobes, reflecting

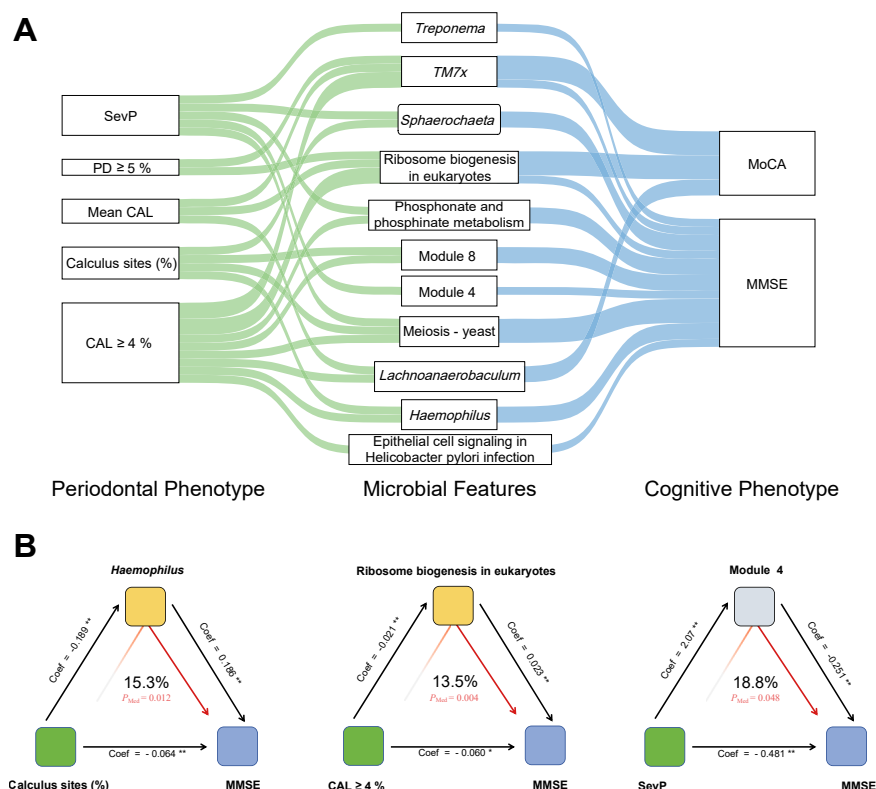


Fig. 5: Mediation analysis of oral microbiome features in the periodontal health–cognition association. Note: (A) Sankey diagram illustrating significant mediation pathways linking periodontal health, oral microbiome features, and cognitive scores (ACME $P < 0.05$). **(B)** Mediation diagram showing *Haemophilus* abundance, ribosome biogenesis in eukaryotes, and Module 4 (*Treponema denticola*, *T. amylovorum*, and *T. medium*) as mediators, with β coefficients and P values along each path (* $P < 0.05$; ** $P < 0.01$). **Abbreviations:** SevP, severe periodontitis; PD $\geq 5\%$, percentage of sites with a probing depth ≥ 5 mm; Mean CAL, Mean clinical attachment loss; Calculus sites (%), percentage of sites with dental calculus; CAL $\geq 4\%$, percentage of sites with clinical attachment loss ≥ 4 mm; MMSE, Mini-Mental State Examination; MoCA, Montreal Cognitive Assessment; ACME, average causal mediation effect.

specific manifestations of the community-wide dysbiosis that has been widely reported to accompany periodontal progression.^{62,63} Notably, although α -diversity did not vary across cognitive groups, β -diversity revealed stratification patterns that paralleled periodontal status, and pathobionts such as *F. nucleatum* and *P. denticola* were enriched in participants with dementia, suggesting that periodontitis-related dysbiosis may provide a mechanistic link to cognitive decline.

Multiple measures of periodontal inflammation were consistently associated with elevated *Treponema* abundance. A *Treponema*-dominated co-abundance module (M4), primarily driven by *T. denticola*, correlated with both greater periodontal severity and lower MMSE scores, highlighting a pro-inflammatory microbial consortium linking oral and cognitive health. Conversely, previous studies have highlighted the critical role of nitric oxide (NO) in neurological and vascular health, pointing to the nitrate-metabolising oral microbiota as potential prebiotic and probiotic targets.^{64–67} We observed that protective effects on cognition were associated with nitrate-reducing genera (*Haemophilus*, *Rothia*),⁶⁸ the anti-inflammatory taxon TM7x,^{69,70} and module M8, which includes *H. parainfluenzae* and *N. subflava*, species harbouring genes for nitrite-to-nitric oxide conversion, a pathway critical for periodontal homeostasis.^{71,72}

Functional profiling highlighted a set of oxidative stress-related features associated with inflammation. Worsening periodontal inflammation was associated with increased representation of bacterial motility and colonisation, such as flagellar assembly and chemotaxis,^{73,74} alongside innate immune activation pathways, including NOD-like receptor signalling, which are closely linked to inflammasome activation and redox-sensitive immune responses.^{75,76} Concurrent reductions in cysteine and methionine metabolism suggested impaired availability of sulphur-containing substrates essential for antioxidant defence and redox buffering.^{77,78} Enhanced AMPK-related stress response signalling,⁷⁹ together with diminished neuroactive signalling pathways such as glutamatergic and GABAergic synapse annotations, further indicated heightened cellular stress and disrupted neural communication.⁸⁰ Consistently, elevated NOD-like receptor signalling and reduced cytochrome P450-reduced detoxification were associated with poorer cognitive performance.⁸¹ Conversely, pathways related to FoxO signalling, which plays a central role in oxidative stress resistance, together with pathways involved in longevity regulation, were positively associated with cognitive scores.^{82,83} Considering the well-established contribution of oxidative stress to AD pathogenesis,^{84–86} our findings suggest that progressive periodontal inflammation may promote systemic oxidative stress and neuroinflammation processes, thereby contributing to cognitive decline.

The progression of periodontitis disrupts the local microenvironment, notably through the remodelling of the junctional epithelium into a deeper pocket epithelium,⁸⁷ creating a nutrient-rich niche that promotes the expansion of inflammation-associated pathobionts and dysbiosis.⁸⁸ In turn, the oral microbiome and its metabolites may mediate chronic inflammation, entering systemic circulation or reaching the brain through neural pathways to trigger neuroinflammatory processes.⁸⁹ Following previous studies,^{90–92} we conducted in silico mediation analysis to explore potential mediating pathways of the oral microbiome. Within the periodontitis–oral microbiota–cognition framework, we identified 11 relevant microbial features, including TM7x, *Haemophilus*, and co-abundance modules M4 and M8, that partially mediated the relationship between periodontal status and cognition. Pro-inflammatory pathogen-dominated modules (e.g., M4) contributed to adverse cognitive outcomes, whereas nitrate-reducing commensals (e.g., M8, *Haemophilus*) exhibited protective associations, with ACME estimates of 18.8% and 15.3%, respectively. These mediating features were linked to functional pathways such as ribosome biogenesis, phosphonate metabolism, inflammatory signalling, and nitrogen metabolism, highlighting potential mechanisms through which oral dysbiosis may influence cognitive processes.^{64,66,93,94} Mechanistically, oral bacteria and their metabolites could modulate systemic inflammation, nitric oxide homeostasis, and blood–brain barrier integrity, thereby shaping neuroinflammatory responses and neuronal function. While causality cannot be inferred from this cross-sectional design, the integration of compositional, functional, and network-level analyses provides a coherent framework supporting a role for the oral microbiome in the periodontal–cognition axis.

Strengths of this study include its large, well-characterised community sample and the integration of detailed oral, microbial, and cognitive phenotyping, coupled with comprehensive multivariable and network analyses. These features enabled a robust exploration of the oral–brain axis in a real-world population setting. The findings highlight the translational potential of preserving periodontal health and modulating oral microbiota as strategies to support cognitive health, although interventional validation is warranted. However, several limitations should be acknowledged. First, the cross-sectional design precludes causal inference, preventing the establishment of temporal relationships among periodontal status, oral dysbiosis, and cognition. Second, 16S rRNA gene sequencing offers limited taxonomic resolution and provides only inferred, rather than directly measured, functional profiles. Finally, although the mediation analyses suggest plausible biological pathways, they are based on biological hypotheses and cross-sectional data, and thus cannot establish causality. These findings should therefore be

interpreted as exploratory, warranting further investigation and validation through future longitudinal and interventional studies. Future research should therefore prioritise longitudinal and interventional designs, as well as multi-omics integration—including inflammatory profiling and neuroimaging—to further elucidate the mechanisms underlying the oral–brain axis.

In summary, based on this large community-based cohort, we found that poor periodontal health was associated with cognitive decline, with alterations in the oral microbiome suggesting a potential mediating role in this relationship. Worsening periodontal status was accompanied by progressive microbial dysbiosis, characterised by enrichment of pro-inflammatory taxa, depletion of nitrate-reducing commensals, and activation of oxidative stress-related functional pathways. Collectively, these findings support a plausible mechanistic link along the oral–brain axis and highlight periodontal health and oral microbial homeostasis as potential targets for early prevention and therapeutic strategies in cognitive impairment.

Contributors

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Writing-review & editing: all authors.

Data curation: X.C., Y.J.

F.L. and X.L. accessed and verified the underlying data. All authors have read and approved the manuscript.

Data sharing statement

The sequencing datasets generated in this study have been deposited in the National Omics Data Encyclopedia (<https://www.biosino.org/node/>) under accession numbers OEP00006862.

The codes were deposited and made publicly available at https://github.com/JW-Lx/Oral-microbiome_Periodontal-health_Cognitive-aging.

Any additional information required to reanalyse the data can be obtained from the lead contact upon reasonable request.

Declaration of interests

The authors declare no conflict of interest.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at <https://doi.org/10.1016/j.ebiom.2026.106231>.

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