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RESEARCH ARTICLE

Periodontal dysbiosis associates with reduced CSF Aβ42 in cognitively normal elderly

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Abstract

Introduction: Periodontal disease is a chronic, inflammatory bacterial dysbiosis that is associated with both Alzheimer's disease (AD) and Down syndrome.

Methods: A total of 48 elderly cognitively normal subjects were evaluated for differences in subgingival periodontal bacteria (assayed by 16S rRNA sequencing) between cerebrospinal fluid (CSF) biomarker groups of amyloid and neurofibrillary pathology. A dysbiotic index (DI) was defined at the genus level as the abundance ratio of known periodontal bacteria to healthy bacteria. Analysis of variance/analysis of covariance (ANOVA/ANCOVA), linear discriminant effect-size analyses (LEfSe) were used to determine the bacterial genera and species differences between the CSF biomarker groups.

Results: At genera and species levels, higher subgingival periodontal dysbiosis was associated with reduced CSF amyloid beta (Aβ)42 ($P = 0.02$ and 0.01) but not with P-tau.

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Discussion: We show a selective relationship between periodontal disease bacterial dysbiosis and CSF biomarkers of amyloidosis, but not for tau. Further modeling is needed to establish the direct link between oral bacteria and Aβ.

KEYWORDS
16S rRNA sequencing, Alzheimer's disease, amyloid, CSF biomarkers, infection, normal aging, oral bacterial dysbiosis, P-tau, periodontitis

1 | INTRODUCTION

The accumulation of amyloid beta (Aβ) plaques and tau neurofibrillary tangle pathology in the brain are the central pathological features of Alzheimer's disease (AD). Brain amyloid deposition is hypothesized to be the first AD pathological feature beginning in the preclinical phase, decades before cognitive dysfunction and preceeding tau tangle accumulations.[1]

The mechanisms by which brain amyloid pathology develops are incompletely understood, as diverse pathways may be at play. Converging evidence points toward inflammation, infections, and bacterial dysbiosis of gut and oral cavity as potential candidates.[2–5]

Periodontal disease (PerioD) is an oral, chronic, inflammatory, dysbiotic bacterial condition affecting more than 50% of elderly people.[6,7] Up to 700 species colonize the subgingival biofilm; among them, several known periodontal bacterial species are enriched in PerioD, including: *Porphyromonas gingivalis*, *Tannerella forsythia*, *Treponema denticola*, *Prevotella spp*, *Porphyromonas endodontalis*, and *Fretibacterium fastidiosum*.[8,9] Other bacteria are enriched in periodontal health including species belonging to *Rothia*, *Corynebacterium*, *Veillonella*, *Actinomyces*, *Streptococcus*, and *Capnocytophaga*.[10–14] Periodontal bacterial dysbiosis is often expressed as the balance between PerioD-associated and health-associated bacteria.[15]

It is well known that periodontal bacteria, particularly in the presence of oral inflammation, gain access to the systemic circulation and can impact distant sites including heart,[16] joints,[17] and liver.[18] Some forms of periodontal bacteria are also associated with cancer.[19] Recent evidence suggests that the brain can also be affected.[20] Murine models of PerioD show that pathogenic periodontal bacteria can induce brain inflammation and Aβ deposition.[5,20,21] We previously reported in an (11C)-PiB-PET (Pittsburgh compound-B positron emission tomography) study of cognitively normal elderly subjects that PerioD was associated with greater brain Aβ load.[22] However, to date, the relationships between the periodontal bacterial composition and biomarker evidence for AD pathology have not been described.

The present cross-sectional study tested the hypothesis that in elderly cognitively normal subjects, dysbiosis of the oral subgingival microbiome is associated with cerebrospinal fluid (CSF) evidence of the AD-signature pathology, which includes Aβ and tauopathy.

2 | METHODS

2.1 | Study subjects and design

Forty-eight subjects participated in this cross-sectional study. From a random community sampling of 250 cognitively normal, healthy elderly subjects, 48 subjects participated in both CSF and dental studies. Consistent with task force recommendations,[23] subjects had standardized examinations that consisted of medical, neurological, psychiatric, neuropsychological, apolipoprotein E (*APOE*) genotyping, and magnetic resonance imaging (MRI) examinations as described.[22]

2.2 | Clinical evaluations

Inclusion criteria: All included subjects had ≥12 years of education and were fluent English speakers. Subjects were defined as cognitively normal based on Clinical Dementia Rating (CDR) = 0, Global Deterioration Scale (GDS) ≤2, and Montreal Cognitive Assessment (MoCA) >26.[24]

Exclusion criteria: Individuals with history/medical conditions that could affect brain structure or function such as clinical or MRI evidence of cortical stroke, hydrocephalus, or intracranial mass; uncontrolled hypertension; diabetes; head trauma with loss of consciousness; any neurodegenerative disease; and chronic depression;[25] or subjects taking anti-inflammatory medications for chronic conditions (eg, nonsteroidal anti-inflammatory drugs [NSAIDS], anti-TNFα [tumor necrosis factor α]), antibiotics, or having periodontal treatment within 3 months of the periodontal evaluation were excluded.

2.2.1 | Measures of periodontal disease

Subjects received a complete oral-periodontal examination[22] encompassing examination of six surfaces of each tooth for probing depth, clinical attachment loss, and bleeding on probing. The Periodontal Inflamed Surface Area (PISA) score[26] was dichotomized into the upper tertial and lower tertials as we described previously.[27] The dental exam also included standardized questionnaires for oral history and oral hygiene behavior.[22]

2.3 | Sample collection and evaluation

2.3.1 | Lumbar puncture, CSF collection, and measurement

CSF was collected by lumbar punctures using a 25-gauge needle guided by fluoroscopy.[25] The CSF biomarker assays were blindly conducted at the Clinical Neurochemistry Laboratory, Gothenburg, Sweden under direction of KB and HZ.[25] CSF levels of Aβ42 and P-tau181 were measured using enzyme-linked immunosorbent assay (ELISA) (Innotest, Fujirebio, Ghent, Belgium)[25] by board-certified technicians using a single batch of reagents. Values were expressed in pg/mL. Apolipoprotein E (*APOE*) genotype was determined using polymerase chain reaction (PCR).

2.3.2 | Biomarker cutoff values

Amyloid-positive (Aβ+) subjects were defined as those with CSF Aβ42 levels <600 pg/mL,[28,29] whereas amyloid-negative subjects (Aβ−) had Aβ42 levels ≥600 pg/mL. In total, 22 subjects were Aβ+ and 26 were Aβ−. The cutoff for CSF P-tau+ was ≥45 pg/mL,[30] yielding 20 P-tau+ and 28 P-tau− subjects.

2.3.3 | Collection of subgingival samples

Subgingival bacterial samples were collected from the four deepest periodontal pockets as previously described.[31] The samples were pooled into one vial and stored at −80°C.

2.4 | Microbiome assessment and analyses

2.4.1 | 16S rRNA amplification and sequencing

We previously published the 16S rRNA methodology used in this study,[32,33] see Supplement S1. Briefly, the subgingival plaque DNA was extracted. Using PCR, the V3-V4 region of 16S rRNA gene was amplified and sequenced and the reads were clustered in operational taxonomic units (OTUs) identifying bacterial ranking. We report our analyses at the genus and species levels.

The main exposure or independent variable was the dysbiotic index (DI), as published in the literature and defined as the abundance ratio at genus level of periodontal (*Treponema*, *Porphyromonas*, and *Tannerella*) to healthy bacteria (*Rothia* and *Corynebacterium*).[20] High versus lower DI was defined by the upper tertial versus the two lower tertials. Based on the cutoff, 15 subjects were high DI (DI+) and 33 subjects were low DI (DI−). We also used bacterial species cluster identified by k-clustering as secondary exposure. A total of 29 subjects belonged to Cluster 1 (periodontal cluster) and 19 belonged to Cluster 2 (healthy cluster).

RESEARCH IN CONTEXT

1. **Systematic review**: The literature was reviewed using traditional (eg, PubMed) sources and meeting abstracts and presentations. Growing evidence implicates periodontal disease and its bacteria in the pathophysiology of Alzheimer's disease (AD). However, it is unclear if one bacterium (ie, *Porphyromonas gingivalis*) or multiple dysbiotic bacteria are contributory to AD pathology. It is unclear if bacterial effects are amyloid specific or affect also neurofibrillary pathology. The existent literature is appropriately cited.
2. **Interpretation**: Our findings showed that dysbiotic periodontal bacteria associated with cerebrospinal fluid (CSF) biomarkers of amyloidosis but not neurofibrillary pathology, leading to the hypothesis that periodontal dysbiotic effects on AD pathology is an early event.
3. **Future directions**: This article proposes additional studies that would clarify the role of periodontal dysbiosis in AD pathogenesis: (1) larger cross-sectional studies confirming our findings; (2) large longitudinal studies to determine the role of bacterial dysbiosis in the progression of amyloidosis and potential role in neurodegeneration; (3) the potential for periodontal treatment effects on AD pathology; and (4) the relationship between periodontal and CSF dysbiosis.

2.5 | Statistical methods

Statistical analyses were performed using IBM SPSS (v26, IBM Corp., Armonk, NY). Continuous data are presented as means and standard deviation (SD) and categorical data as percentages. To evaluate biomarker group differences for continuous variables, *t* test and Mann-Whitney *U* (MWU) test were used, whichever was appropriate. For categorical variables, chi-square tests were used. A log transformation was used to normalize the distributions for DI. For microbiome analyses, we used the linear discriminant effect size analysis (LEfSe), which uses an algorithm that combines the statistical modeling with biological significance to reveal biomarker clusters.[34] Effect size (LDA = linear discriminative analysis scores expressed in $\log_{10}$) provides an estimation of the magnitude of the observed effect. In our analyses we used settings of both $P < 0.05$ and LDA ≥2.[34] A k-clustering technique was also performed using the abundance of periodontal and health-associated bacteria discovered in LEfSe.

A number of potential confounders were tested in biomarker prediction models, including age, gender, *APOE* genotype, declarative memory performance (Logic2 of Wechsler Memory Scale-Revised test[9]), education, obesity (BMI), behavior (smoking, brushing, flossing, dentist visits), cardiovascular factors (hypertension, heart disease).

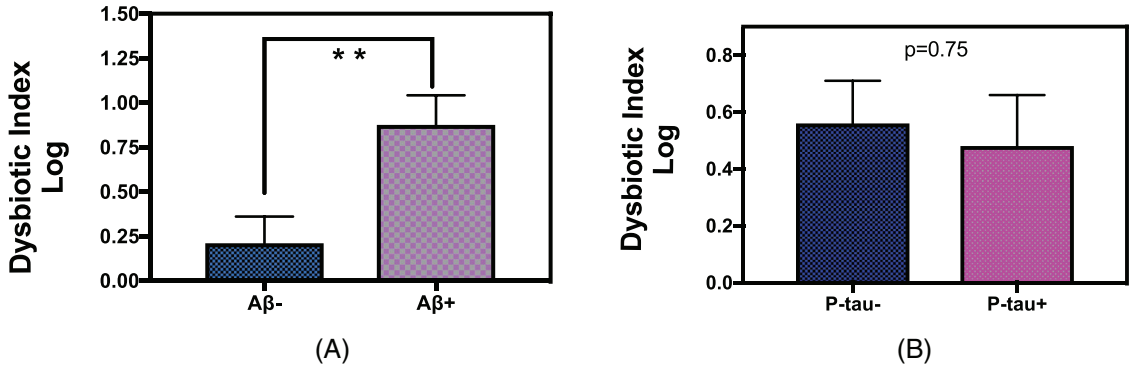

FIGURE 1 Dysbiotic index in Amyloid (A) and P-tau (B) groups. ANCOVA showed that Dysbiotic Index was statistically significant higher in Aβ+ group compared to the Aβ- group and this result maintained the significance after adjustment for *APOE*. However, Dysbiotic Index was not statistically significant between the P-tau groups. Aβ- = amyloid-: CSF Aβ42 ≥ 600 pg/mL; Aβ+ = Amyloid+: CSF Aβ42 < 600 pg/mL. ** = $P < 0.01$. P-tau- = CSF P-tau ≤ 45 pg/mL; P-tau+ = CSF P-tau > 45 pg/mL. Means and SE are presented

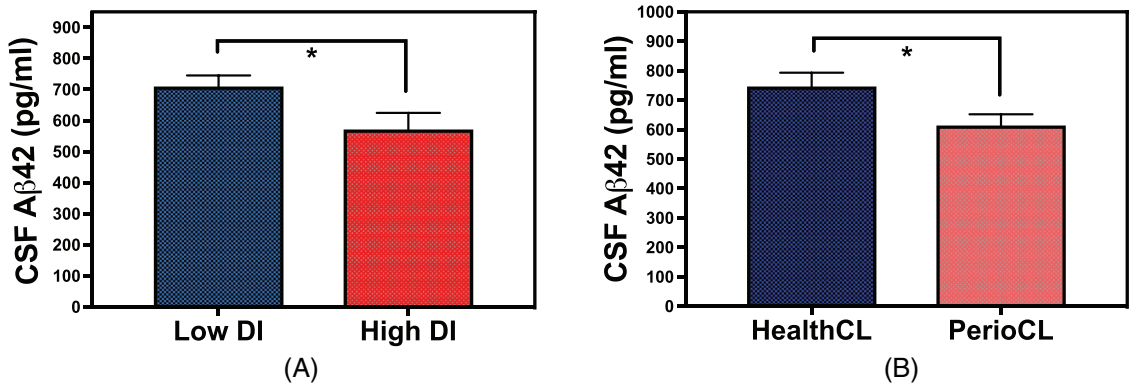

FIGURE 2 CSF Aβ42 in Dysbiotic groups. A: There was a statistically significant difference in the CSF Aβ42 between Low DI (n = 33) and High DI (n = 15) and B: Healthy cluster (HealthCL, n = 19) and periodontal-associated cluster (PerioCL, n = 29) after adjustment for *APOE* ($P = 0.037$ and 0.035 respectively). Means and SE are presented. * = $P < 0.05$

Because only *APOE* was significant, this was routinely used in the analyses. ANOVA and ANCOVA were used to test the differences in continuous variables between CSF biomarker and bacterial groups, respectively. Logistic regression was used to estimate the odds ratios for the association between the dysbiotic groups (DI/microbial cluster) and biomarker group membership. Given the exploratory nature of this study, an unadjusted $P < .05$ level of significance was used.

3 | RESULTS

3.1 | Characteristics of the population

Table 1 presents the characteristics of the study group.

Overall, comparison of CSF biomarker+ (Aβ+, T-tau+) with CSF biomarker− (Aβ−, T-tau−) subjects showed no significant differences in gender, education, BMI, smoking or medical history, or in the number of systemic conditions, declarative memory performance, and time between the periodontal exam and CSF collection (Supplementary Table 1S and 2S). A higher proportion of *APOE* ε4 carriers was found in Aβ+ groups.

3.2 | Dysbiotic index associations with AD biomarkers

Aβ42: In the analysis of covariance (ANCOVA) model (Figure 1A) we found after adjusting for *APOE* that Aβ+ subjects had significantly higher DI scores compared to Aβ− subjects (mean (SEM): Aβ+ = 0.88 [0.17] vs Aβ− = 0.2 [0.15], F[1,45] = 8.05; $P = 0.01$). No interaction was present between *APOE* and Aβ groups on the DI ($P > 0.05$). We also tested if the relationship exists between the dichotomized DI and the CSF Aβ42 levels. After adjusting for *APOE*, the DI+ subjects had significantly lower CSF Aβ42 levels compared to DI− subjects (means [SE]: 571.6 [53.5] and 709.6 [35.9], respectively, $P = 0.04$) (Figure 2A). With *APOE* in the model, there was a significant association between continuous DI and Aβ group membership (OR = 3.96, 95% CI [1.31-11.94], $P = 0.02$). Subjects with CSF Aβ42 values close to the threshold may

TABLE 1 The characteristics of the study group population (n = 48)

Demographic	
Age (Mean [SD])	69.2 (7.9)
Gender n (%)	
Female	26 (54.2)
Education (Mean [SD])	17.8 (2.4)
Behavior	
Smoking (%)	
Yes	3 (6.3)
Oral health behavior	
Brushing (%)	
≤Once/day	11 (22.9)
>1/day	37 (77.1)
Flossing (%)	
Never/rarely	13 (27.1)
>once/week	35 (72.9)
Last dentist visit (%)	
<1 year	43 (87.5)
≥1 year	5 (12.5)
Systemic health	
BMI (Mean [SD])	26.6 (5.1)
Syst. Cond (Mean [SD])	1.06 1.02
Hypertension (%)	
Yes	21 (43.8)
Heart (%)	
Yes	13 (27.1)
Cancer (%)	
Yes	9 (18.8)
Logic2 (Mean [SD])	0.1 (1.1)
ApoE4ε (%)	
Non-Carriers	26 (54.2)
Carriers	22 (45.8)
Perio-CSF (Mean [SD])	1.9 (1.4)
PISA (%)	
Yes	17 (35%)

Abbreviations: Syst. Cond, systemic conditions; Perio-CSF, time in years between periodontal exam and the lumbar puncture; PISA, periodontal inflamed surface area.

be misclassified due to measurement error. We repeated the analyses after excluding subjects with CSF Aβ42 values within 5% of the threshold. The results were similar. We found that after adjusting for *APOE*, Aβ+ subjects (CSF Aβ42 ≤570 pg/mL, n = 18) had significantly higher DI scores compared to Aβ− subjects (CSF Aβ42 ≥630 pg/mL, n = 24) (Mean [SEM]: Aβ+ = 0.78 [0.18] vs Aβ− = 0.19 [0.15], F[1,39] = 5.7; $P = 0.02$).

P-Tau181: In the ANCOVA models, the DI score did not differ between CSF P-tau+ and P-tau− subjects (Mean [SEM]: P-tau+ = 0.56 0.18) vs P-tau− = 0.48 (0.15), (F[1,45] = 0.10; $P = 0.75$); see Figure 1B). No interaction between tau biomarker groups and *APOE* on the DI was found.

3.3 | Subgingival bacteria in the AD biomarkers of amyloid and neurofibrillary pathology

To confirm the differences in subgingival bacteria between CSF biomarker groups, we assessed the microbial abundance at two phylogenetic levels: genus and species. We tested several classic and newly discovered periodontal disease (PerioD)—associated genera (*Treponema*, *Porphyromonas*, *Tannerella*, *Fusobacterium*, *Prevotella*, *Fretibacterium*, *and Dialister*) and several periodontal health-associated genera (*Rothia*, *Corynebacterium*, *Actinomyces*, and *Capnocytophaga*).[10,13,35] In our analyses, we found that (Figure 2S), *Fretibacterium* (%Mean [SD]: Aβ+ = 2.4 [2.5] vs Aβ− = 1.1 [1.7], $P = 0.02$), *Prevotell*a (%Mean [SD]: Aβ+ = 15.1 [8.1] vs Aβ− = 10.0 [6.5], $P = 0.03$), and *Dialister* (%Mean [SD]: Aβ+ = 3.5 [2.6] vs. Aβ− = 1.9 [1.7], $P = 0.01$) were increased, whereas *Corynebacterium* (%Mean [SD]: Aβ+ = 1.1 [1.0] vs Aβ− = 3.1 [2.8], $P = 0.01$), *Actinomyces* ([%Mean [SD]: Aβ+ = 1.0 [1.0] vs Aβ−- = 1.6 [1.6], $P = 0.03$) and *Capnocytophaga* (%Mean [SD]: Aβ+ = 3.8 [3.0] vs Aβ− = 6.6 [4.1] [$P = 0.01$]) were decreased in the Aβ+ group. For the P-tau groups, there were no differences in these genera between P-tau+ and P-tau− ($P > 0.05$).

LEfSe analysis identified candidate bacterial species associated with the AD biomarkers. A total of 16 species were enriched in the Aβ+. They included *Prevotella oris*, *Prevotella denticola*, *Porphyromonas endodontalis*, and *Fretibacterium fastidiosum and Fretibacterium sp HMT 362*, known for their association with PerioD.[14] A total of 12 species were enriched in the Aβ− group (see Figure 3A), including *Corynebacterium matruchotii*, *Corynebacterium durum*, *Capnocytophaga leadbetteri*, and *Actinomyces spp. HMT 175* and *HMT-169* known for their association with periodontal health. To illustrate the difference in relative bacterial abundance and pattern consistency between Aβ+ and Aβ− groups, Figure 3B and 3C show the histogram of raw data representing the bacterial abundance in each subject for one PerioD-associated species, *Fretibacterium fastidiosum* and one health-associated species, *Corynebacterium matruchotii*. Although, *Porphyromonas gingivalis* abundance was not statistically significant between Aβ groups, its histogram showed a pattern comparable to that of *Fretibacterium fastidiosum* (Figure 4S).

Next, we aimed to classify study subjects into groups expressing similar bacterial composition pattern at a species level. Using k-clustering with the abundances of periodontal and health-associated bacterial species described above, we defined two bacterial clusters: Cluster 1 and Cluster 2. Bacterial species contributing to Cluster 1 were periodontal-associated bacteria (*Prevotella oris*, *Prevotella denticola*, *Porphyromonas endodontalis*, and *Fretibacterium fastidiosum and Fretibacterium sp. HMT 362*), whereas those contributing to Cluster 2 were health-associated bacteria *Corynebacterium matruchotii*, *Corynebacterium durum*, *Capnocytophaga leadbetteri*, and *Actinomyces spp. HMT 175* and *HMT-169)*. Thus 29 subjects belonged to Cluster 1

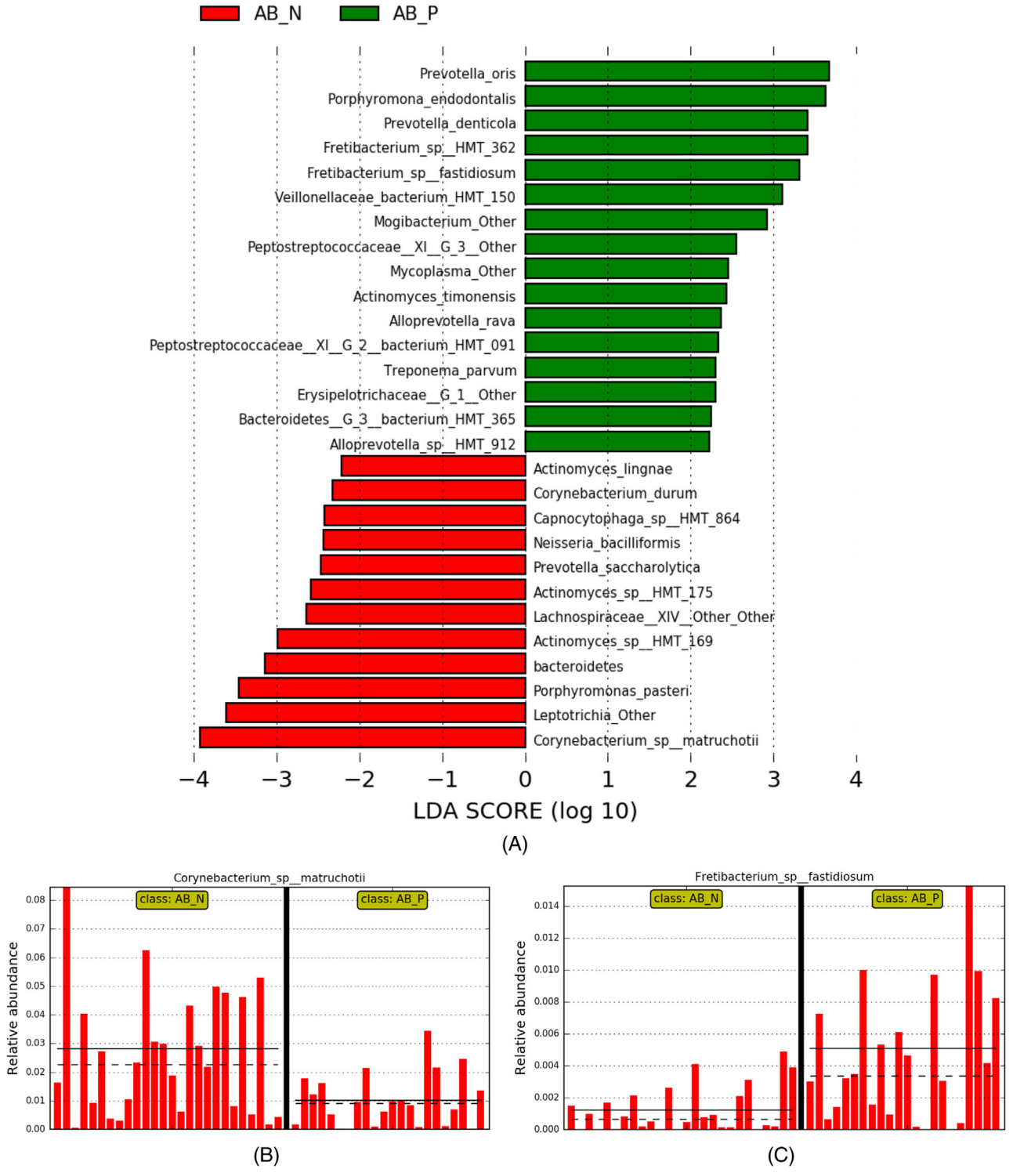

FIGURE 3 Linear Discriminant Analysis (LDA) Effect Size (LEfSe) plot showing species relative abundance in amyloid- (AB_N) and amyloid+ (AB_P) groups. Lefse ($P = 0.05$ and LDA threshold = 2) shows that subgingival bacteria of amyloid+ subjects were enriched in species associated with periodontal disease while amyloid-subjects were enriched in species associated with periodontal health (3A). Horizontal bars (red = AB_N; green = AB_P) represent the effect size for each specie. The LDA scores represent the log10 transformed LDA score. Negative as well as positive values denotes increased in abundance compared to the other group. Consistency in the relative abundance and pattern of the health associated bacterium *Corynebacterium matruchotii* and periodontal associated bacterium *Fretibacterium fastidiosum* are shown in Figure 3B and 3C. Each red bar of the histograms represents the relative abundance of one subject. The vertical thick black bar divides the amyloid- (AB_N) from amyloid+ (AB_P) subjects. The solid and dotted horizontal black lines indicate the mean and median relative abundance values for each group, respectively

and 19 belonged to Cluster 2. In the ANCOVA model (Figure 2B) with CSF Aβ42 as the dependent variable, we found after adjusting for *APOE* that Cluster 1 had significantly lower CSF Aβ42 compared to Cluster 2 (Mean [SEM]: Cluster 1 = 614.1 [38.2] vs Cluster 2 = 746.4 [47.2], F[1,44] = 4.73; $P = 0.04$). No interaction was present between *APOE* and cluster groups on the CSF Aβ42 ($P > 0.05$). However, there was a significant association between bacterial clusters and Aβ group membership (OR = 17.50, 95% CI [2.84-107.90], $P = 0.002$).

For P-tau, LEfSe analysis showed three species enriched in P-tau+, group whereas five species were enriched in P-tau− group (Figure 3S). Among these species, *Porphyromonas catoniae* has been reported in periodontal health. Clustering of bacteria associated with P-tau was not feasible because one cluster had only one subject.

4 | DISCUSSION

4.1 | Main findings

To our knowledge, this is the first report of an association between subgingival periodontal bacteria and CSF biomarkers of AD pathology in cognitively normal elderly people. We found that subgingival periodontal dysbiosis characterized by increases in periodontal-associated bacteria and decreases in health-related bacteria associated with reduced CSF Aβ42 but not with CSF P-tau.

4.2 | Periodontal bacterial dysbiosis and brain amyloidosis

The genera level. The DI composed of the genera ratio of harmful periodontal bacteria (*Treponema*, *Porphyromonas*, *Tannerella*) to healthy bacteria (*Rothia*, *Corynebacterium*)[15] was increased in Aβ+ group. A higher DI conferred a four-fold likelihood of belonging to Aβ+ group. Specific analysis of the bacterial composition from our sample showed at the genera level that periodontal *Fretibacterium*, *Prevotell*a, and *Dialister* were increased in Aβ+ group, whereas healthy *Corynebacterium*, *Actinomyces*, and *Capnocytophaga* were increased in Aβ− group.

The species level. LEfSe showed that the Aβ+ group was enriched in subgingival PerioD bacteria such as *Prevotella oris*, *Prevotella denticola*, *Porphyromonas endodontalis*, and *Fretibacterium fastidiosum and Fretibacterium sp. HMT 362*, whereas the Aβ− group was enriched in periodontal health-related bacteria such as *Corynebacterium matruchotii*, *Corynebacterium durum*, *Capnocytophaga leadbetteri*, and *Actinomyces spp. HMT 175* and *HMT-169*. Furthermore, we find that being in periodontal bacterial Cluster 1 conferred a high likelihood of belonging to Aβ+ group.

The association between subgingival bacterial dysbiosis and Aβ groups was consistent whether we used DI at the genera level or clustering at the species level. *Porphyromonas endodontalis* and *Treponema parvum* enriched in Aβ+ group and *Corynebacterium matruchotii* and *Corynebacterium durum* enriched in Aβ− group contributed to DI. Both results point toward the significance of healthy bacteria in Aβ−. Other species less known for their association with PerioD/health were also differentially enriched in the amyloidosis groups.

The association of periodontal bacteria and AD pathogenesis has been studied previously (review [5]). Animal studies of PerioD/infections showed brain pathology including neuroinflammation, amyloid accumulation, tau pathology, and neurodegeneration. Most animal models used *Porphyromonas gingivalis* or its lipopolysaccharides as the inducing agent. Dominy et al.[20] showed that oral *Porphyromonas gingivalis* induced brain colonization and AD pathology, and that these effects were reduced by inhibiting gingipain, a *Porphyromonas gingivalis* virulence factor. In our study, *Porphyromonas gingivalis* was increased in the Aβ+ group, but did not reach statistical significance. Larger sample sizes may show its enrichment. Our study suggests the involvement of other periodontal bacteria in Aβ pathology.

Our prior PiB-PET study demonstrated increased Aβ in normal subjects with PerioD.[22] The mechanisms by which periodontal-associated bacteria can influence brain amyloid are multiple.[5] Bacteria can reach the brain via systemic circulation or nerve pathways and directly induce pathologic changes in brain (ie, amyloid synthesis or clearance, synaptic dysfunction). For example, *Treponema* species were detected in the trigeminal ganglia.[36] Bacteria can modulate local and systemic inflammation that in turn contribute to brain inflammation and amyloid pathology. *Prevotella* species and *Porphyromonas gingivalis* have been associated with a proinflammatory response.[37] Periodontal bacteria could also seed the gut and thus influence AD pathology.[18]

Dysbiosis or normobiosis is defined by the composition and functional make-up of the whole bacterial community and not by one bacterium.[38] Each individual microbiome composition is complex and can vary in the balance between pathogenic and healthy bacteria.[39] Although, *Porphyromonas*, *Treponema*, and *Tannerella* are defined as classic periodontal pathogens, many studies have shown that their prevalence is not consistent in PerioD. Moreover, the virulence factors contributing to the dysbiotic community are upregulated in many other bacteria.[39,40] *Porphyromonas gingivalis* is expressed in 65% to 85%[41] of those with PerioD and in 10% to 40% of healthy subjects.[41,42] Therefore, the association of other pathogenic bacteria in PerioD is common and a complex association with Aβ+ can be expected.

The role of healthy subgingival bacteria in AD pathogenesis has not been studied. Our study points toward these bacteria as having a role in brain Aβ homeostasis. Although this result is novel as it relates to oral dysbiosis, a similar association has been described in the gut. Several interpretations can be put forward to explain our results. High levels of healthy bacteria maintain bacterial balance; they decrease subgingival and therefore systemic inflammation; and contribute to the nitrite oxide production known for its health effects.[43] They can inhibit the production of virulent factors by other bacteria and reestablish bacterial balance. In this environment, fewer pathogenic bacteria would escape the subgingival environment and travel to the brain. Healthy bacteria may also access the circulation and brain and exert anti-inflammatory effects there. For example, *Actinomyces* associate with high anti-inflammatory host response.[44] The role of healthy bacteria has also been demonstrated in head and neck cancer[19] in which *Corynebacterium* may be protective.

4.3 | Periodontal bacterial dysbiosis and neurofibrillary pathology

At the genera level, DI or specific bacterial genera did not associate with the tau pathology biomarker CSF P-tau. Although, *Porphyromonas catoniae* showed enrichment in P-Tau- group, the histogram pattern was inconsistent questioning this association. Among animal studies investigating the role of PerioD/dysbiosis in AD pathology only a few studies presented evidence for neurofibrillary pathology.[5,45] The lack of consistent association between PerioD and tau pathology in this study and others may relate to disease course and timing of assessment. These results beg for longitudinal study to learn the degree and timing of AD pathology with respect to PerioD.

4.4 | Strengths and weaknesses

Our sample is quite homogeneous composed of cognitively normal, educated, with good systemic health and oral habits. All medical, neuropsychological, imaging, CSF collection and dental exams were standardized. One trained periodontist performed all periodontal evaluations blind to CSF collection.

There are several limitations related to our study that include the cross-sectional design, population characteristics and sample size. The cross-sectional design of our study does not allow inference regarding causation. The number of subjects in this study is relatively small given the number of variables under consideration. Therefore, the confidence intervals were large and the point estimate imprecise.

An additional bias is the lack of generalizability of the sample to a larger population. Although, our sample was derived from the community, the participants were highly screened and self-selected. Notably, 95% of our subjects were white and 42% were *APOE* ε4 carriers.

In conclusion, we showed that measures of periodontal bacterial dysbiosis were associated with lower CSF biomarkers of amyloidosis. Of additional importance, our results point to both pathogenic and healthy bacteria in modulating CSF Aβ42 levels. Periodontal dysbiosis can be changed with treatment, thereby offering hope that Aβ accumulation may be prevented, slowed, or even reversed.

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CONFLICTS OF INTEREST

Kaj Blennow has served as a consultant, at advisory boards, or at data monitoring committees for Abcam, Axon, Biogen, JOMDD/Shimadzu. Julius Clinical, Lilly, MagQu, Novartis, Roche Diagnostics, and Siemens Healthineers, and is a co-founder of Brain Biomarker Solutions in Gothenburg AB (BBS), which is a part of the GU Ventures Incubator Program. Henrik Zetterberg has served at scientific advisory boards for Denali, Roche Diagnostics, Wave, Samumed, Siemens Healthineers, Pinteon Therapeutics and CogRx, has given lectures in symposia sponsored by Fujirebio, Alzecure and Biogen, and is a co-founder of Brain Biomarker Solutions in Gothenburg AB (BBS), which is a part of the GU Ventures Incubator Program (outside submitted work). No conflict of interest is reported for any of the other authors.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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