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Brain age mediates gut microbiome dysbiosis-related cognition in older adults

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Abstract

Background Recent studies have focused on improving our understanding of gut microbiome dysbiosis and its impact on cognitive function. However, the relationship between gut microbiome composition, accelerated brain atrophy, and cognitive function has not yet been fully explored.

Methods We recruited 292 participants from South Korean memory clinics to undergo brain magnetic resonance imaging, clinical assessments, and collected stool samples. We employed a pretrained brain age model– a measure associated with neurodegeneration. Using cluster analysis, we categorized individuals based on their microbiome profiles and examined the correlations with brain age, Mental State Examination (MMSE) scores, and the Clinical Dementia Rating Sum of Box (CDR-SB).

Results Two clusters were identified in the microbiota at the phylum level that showed significant differences on a few microbiotas phylum. Greater gut microbiome dysbiosis was associated with worse cognitive function including MMSE and CDR-SB; this effect was partially mediated by greater brain age even when accounting for chronological age, sex, and education.

Conclusions Our findings indicate that brain age mediates the link between gut microbiome dysbiosis and cognitive performance. These insights suggest potential interventions targeting the gut microbiome to alleviate age-related cognitive decline.

Keywords Brain age, Gut microbiome, Cognition

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Background

The human gut microbiome, a complex and dynamic ecosystem of microorganisms, plays a vital role in maintaining host health and influencing disease progression. Advances in sequencing technologies and bioinformatics have uncovered the intricate relationships between microbial communities and the host's metabolic, immunological, and neurological systems. Central to this understanding is the concept of the "gut–brain axis," a bidirectional communication network linking the enteric and central nervous systems (CNS) through neural, endocrine, immune, and humoral pathways. Through these mechanisms, the gut microbiome has been hypothesized to affect brain development, behavior, and cognitive function [1, 2].

Emerging research suggests that gut microbiome dysbiosis—a state of microbial imbalance—is associated with accelerated gray matter aging. Dysbiosis has been linked to inflammation and increased intestinal permeability, leading to systemic and neural inflammation that can negatively impact cognitive function [3]. Aging appears to exacerbate these changes, marked by decreased diversity in beneficial microbial species, such as anti-inflammatory *Bifidobacterium*, and increased prevalence of pro-inflammatory species like *Enterococcus* [4–6]. These microbial shifts, coupled with reduced immunological function and heightened release of inflammatory products, may further accelerate brain aging, contribute to cognitive decline, and even promote amyloid and tau deposition associated with Alzheimer's disease [7].

Despite numerous studies on the connection between the gut microbiome and neurodegenerative disease, few human studies have clearly shown a direct link between neurodegenerative markers and specific gut microbiome dysbiosis [8–12]. To explore this further, we focused on brain age and data-driven clustering of gut microbiome dysbiosis. Brain age, a biomarker derived from neuroimaging data, reflects the cumulative impact of environmental stressors, modifiable risk factors, and neurodegenerative processes [13]. In previous research, we used machine learning to estimate brain age from gray matter volume and demonstrated its potential for predicting future cognitive decline [14–17]. Recent studies emphasize the utility of clustering techniques in analyzing gut microbiome dysbiosis [18]. For example, Saji et al. identified distinct microbiome clusters that differentiated individuals with dementia from healthy controls, showing significant differences in brain imaging across these clusters [19]. Although dysbiosis has been reported in Alzheimer's Disease (AD) patients, its direct role in accelerated brain aging remains unverified [20].

Therefore, we aimed to explore the relationship between gut microbiome composition, brain aging, and cognitive function. Using cluster analysis, we categorized individuals based on their microbiome profiles and examined the correlations with brain age, the Mini-Mental State Examination (MMSE) score, and the score of Clinical Dementia Rating Sum of Boxes (CDR-SB), which measures cognitive function and dementia severity in older individuals. We additionally explored AD brain imaging and blood biomarkers as mediators in the relationship between microbiome changes and cognitive function.

Methods

Participants

This study was a part of the ongoing Biobank Innovations for chronic Cerebrovascular disease With ALZheimer's disease Study (BICWALZS) and the Center for Convergence Research on Neurological Disorders. BICWALZS was initiated in 2016 by the National Biobank of Korea and the Ajou University School of Medicine [21]. The original goal was to facilitate, regulate, and ensure the optimal use of human biological specimens for research using real-world data on subjective cognitive decline (SCD), mild cognitive impairment (MCI), AD, and subcortical vascular dementia (SVaD). BICWALZS is registered in the Korean National Clinical Trial Registry (Clinical Research Information Service; identifier: KCT0003391; Registration Date: 2018/07/04; http://cris.nih.go.kr/cris/en/use_guide/cris_introduce.jsp). This study was approved by the Institutional Review Board of Ajou University Hospital (AJOUIRB-SUR-2021-038). Written informed consent was obtained from all the participants and their caregivers. The participants from the BICWALZS were recruited from the memory clinics of seven university-affiliated hospitals and community geriatric centers in South Korea. However, we included participants who provided stool samples ($N = 292$). All participants were recruited from two sites: a memory clinic affiliated with the Ajou University Hospital and the Suwon Community Geriatric Mental Health Center. All participants were Koreans of Eastern Asian ethnicity. None of the participants in this study were part of the initial brain age training sample of our previously trained model [16]. In total, we analyzed cross-sectional data from 292 participants, including information from brain MRI, amyloid PET, APOE genotyping, CDR scores, clinical diagnoses, and blood laboratory assessments.

Clinical and biological assessment

Our research team has been managing a clinical research registry since 2005 and initiated this cohort in 2016 based on the subject recruitment criteria established at that time [22]. The clinical diagnosis criteria used for this study were as follows: SCD criteria included self- and/or informant reports of cognitive decline but no objective impairment in cognitive tasks (no less than − 1.5

SD in each of the neurocognitive test domain and Clinical Dementia Rating [CDR] = 0) [23]. Patients with MCI were evaluated based on a CDR score of 0.5 and the expanded Mayo Clinic criteria [24, 25]. Patients with AD dementia were evaluated using the National Institute on Aging-Alzheimer's Association core clinical probable AD dementia criteria [26]. SVaD was evaluated based on above-moderate white matter hyperintensity (WMH) and vascular dementia criteria, following the Diagnostic Statistical Manual of Mental Disorders, fifth edition [27]. Patients with a history of neurological or medical conditions, such as territorial cerebral infarction, intracranial hemorrhage, Parkinson's disease, heart failure, renal failure, or other medical conditions that could interfere with the study, were excluded. General cognitive function was evaluated using the MMSE [28]. Dementia severity was measured using the CDR-SB scores [24]. Cognitive function was assessed using the Seoul Neuropsychological Screening Battery, a standardized neuropsychological test that evaluates language, visuospatial abilities, memory, and executive functions [29].

For the laboratory assessments, morning blood samples were collected via venipuncture after an overnight fast. Samples were drawn into serum separation and dipotassium ethylenediaminetetraacetic acid tubes. Baseline blood tests included complete blood count, blood urea nitrogen, creatinine, albumin, liver function tests, fasting serum glucose, glycated hemoglobin, serum lipids, total protein, folic acid, high-sensitivity C-reactive protein, fibrinogen, venereal disease research laboratory test, Treponema pallidum hemagglutination, electrolyte analysis, vitamin B12, homocysteine, thyroid function tests, and apolipoprotein E (APOE). Along with laboratory assessments, surveys were conducted to gather basic psychosocial status (lifetime alcohol consumption, smoking, nutrition, depression, anxiety and sleep) and medical histories (hypertension, hyperlipidemia, and other past physical illnesses) [21].

Microbiome

Stool samples were collected at the Ajou University Hospital biobank the day before the clinical assessment and within two weeks of the brain MRI, using a sterilized stool container, and were stored at -20 °C until further processing. Bacteria within the stool were purified, and DNA was extracted for sequencing.

The extracted bacterial 16s rDNA from each sample was assigned a unique barcode sequence for library preparation. Next-Generation Sequencing was then conducted on all the isolated bacterial 16s samples using the Illumina MiSeq platform technology [30]. After all microbiome samples were sequenced, taxonomic profiling using SILVA DB and downstream analyses were conducted [31].

DNA extraction was performed by using FastDNA Spin Kit for Soil (MP Biomedicals, Irvine, California, USA) on stool samples. Polymerase chain reaction (PCR) was then performed to amplify template out of the DNA samples by using V3-V4 region primers with overhang adapters attached, which were 16S_V3_F (5′- TCG TCG GCA GCG TCA GAT GTG TAT AAG AGA CAG CCT ACG GGN GGC WGC AG-3′) and 16S_V4_R (5′-GTC TCG TGG GCT CGG AGA TGT GTA TAA GAG ACA GGA CTA CHV GGG TAT CTA ATC C-3′). After attaching Nextera® XT Index Kit V2, an Illumina adapter primer, sequencing was performed using an Illumina V3 600 cycle cartridge and Illlumina MiSeq equipment (San Diego, California, USA). The FASTA files were collected and analyzed using quantitative insights into microbial ecology-2 (QIIME2) software package (version 2022.2) [30]. The Demux plugin was utilized to decompress the fasta files, and the DADA2 pipeline was employed for sequence quality control and feature table construction to generate a unique sequence file (version 1.18) [31]. Sequences with forward and reverse median quality scores below 30 and sequence sizes shorter than 126 were filtered out during the noise reduction filtering process in DADA2. Taxonomy was assigned using the Silva database (version 138.1) [32]. The 16 S ribosomal RNA sequence database (https://ftp.ncbi.nlm.nih.gov/blast/db/) of NCBI's basic local alignment search tool (BLAST) + was used for sequence alignment to assign classifications to the unique sequences called operational taxonomic unit (OTU) [33]. Additionally, Silva shares the most taxonomic units with NCBI [34]. After denoising, the OTU counts and taxonomy were merged into a single table. The relative abundance to normalize their counts was used to identify microbial community types.

APOE genotyping and measurement of amyloid deposition

Detailed methods used for APOE, plasma markers (amyloid Aβ40, Aβ42, Aβ42/40 and p-tau 217) and amyloid PET standard uptake value ratio (SUVR) are provided in Supplementary Methods 1–3.

Magnetic Resonance Imaging (MRI) analysis and brain age estimation

Detailed methods used for MRI acquisition, structural MRI processing, brain age estimation and MRI sequence parameters are provided in Supplementary Methods 4 and Supplementary Table 1.

We utilized a previously validated brain age estimation algorithm designed to reflect neurodegenerative changes in gray matter. This algorithm employs whole-brain, voxel-wise gray matter volume data and has been validated in a large training set of healthy adults [16]. Additionally, it has been independently tested and proven reliable within this cohort, demonstrating

its ability to capture age-related brain changes effectively [14]. We used the brain age residual as a measure of age-related brain health. It represents the error term remaining after adjusting brain age for chronological age, age squared, and sex. This is expressed by the equation: brain age = intercept + β1[age centered] + β2[age centered squared] + β3[sex] + brain age residual. A higher brain age residual indicates that the brain is older than expected for the individual's chronological age after accounting for these factors.

Clustering microbial community types

Based on the bacterial abundance, we used the Dirichlet multinomial mixtures (DMMs) algorithm to identify microbial community types [35]. The DMM was applied to 16 S rRNA gene sequencing, and the microbiota composition was analyzed at the phylum level. The DMM can efficiently cluster samples based on the relative abundance of the identified microbiota. Based on the lowest Laplace approximation index, appropriate microbial community type numbers (DMM clusters) were determined. The DMM methodology effectively manages the large data dimensionality associated with microbiome analyses. This facilitated the use of sequencing results, including bacterial community types and clinical variables, for multivariable analyses. Detailed methods used for DMM are provided in Supplementary Methods 5. The analyses described above were performed using the R package Dirichlet Multinomial v1.36.0.

Factors associated with cluster groups

To understand what factors were associated with cluster groupings, we conducted elastic net regression on the groups (as outcome) with the following sets of features: sex, education, chronological age, brain age_residual, body mass index, global amyloid standardized uptake value, the MMSE score, the CDR-SB score, neurocognitive test results (Digit span test, Boston Naming Test, Rey complex figure test, Seoul verbal learning test delayed recall test, and Controlled Oral Word Association Test), psychological and behavioral symptoms (Beck Anxiety Inventory, Montgomery–Asberg Depression Rating Scale, Pittsburgh Sleep Quality Index, and alcohol consumption), blood test results (hemoglobin, hematocrit, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, mean corpuscular volume, blood urea nitrogen/creatinine, thyroid stimulating hormone, free thyroxine, albumin, total cholesterol, triglycerides, high-density lipoprotein [HDL], low-density lipoprotein, fasting glucose, HbA1c, vitamin B12, and homocysteine), history of hypertension, diabetes, thyroid disease, brain MRI WMH, lacune and intracranial volume, APOE status, blood amyloid and tau level, and study site. The factors analyzed—clinical assessments, brain imaging, and blood markers—were integral components of the BIC-WALZS cohort. These elements were included in the cohort's evaluation items due to their association with neurodegenerative diseases such as AD with cerebrovascular component [21]. We conducted multiple imputations (five imputations) with all features included, using the mice package in R with the random forest approach. We then conducted an elastic net regression using the eNetXplorer package in R. We included all the features listed above to predict the cluster groupings. We employed five-fold cross-validation, optimizing α values from 0 to 1 and 100 λ values. This process was repeated 500 times, and the cluster groups were then permuted with 250 permutations to estimate null bounds. We then used these to estimate the *p*-values for the models and each variable. We chose the model with the best performance and lowest regularization (e.g., α closest to 0). The *p*-values were then combined using Fisher's method [36–38].

Relationship between gut microbiome composition, brain age and cognitive function

Mediation analyses were performed to assess whether brain age mediated the relationship between the microbiome cluster and MMSE scores or between the cluster and CDR-SB scores in the full sample. Exploratory analyses were additionally conducted to examine the mediation effects of Alzheimer's disease markers, including amyloid SUVR, plasma amyloid Aβ40, Aβ42, Aβ42/40, and p-tau 217, as well as other clinical factors identified as significant in the elastic net model. Mediation analyses were conducted using the R package lavaan v0.6-11 [39]. We calculated 95% bias-corrected and accelerated bootstrap confidence intervals for indirect effects using at least 5,000 bootstrap samples. Confidence intervals excluding zero indicated significant bootstrapped mediation effects. Our model aimed to assess the effect of microbiome clusters on the MMSE and CDR-SB scores, mediated by brain age. The same bootstrap method was used to calculate confidence intervals. Several indexes were calculated to evaluate the model fit to the data: root mean square error of approximation (RMSEA), adjusted goodness-of-fit index (AGFI), comparative fitting index (CFI), and normed fit index (NFI) [40]. Statistical significance was evaluated at a 5% significance level ($p < 0.05$), and all analyses were performed using R (version 4.1.0) and its open-source statistical packages.

Additionally, Exploratory linear regression was conducted to examine the association between microbiome composition and brain age at a lower taxonomic level than that used in the mediation analysis, focusing on certain phyla that distinguished the two groups.

Results

Demographic characteristics

In total, 288 patients with normal cognitive function, SCD, MCI, AD, or SVaD were included. One patient was excluded due to missing microbiome data, and three patients were excluded from the analysis because of noise caused by movement in the MRI images. Among them, 281 had amyloid PET data, and 150 had Aβ40 data. The characteristics of the samples are listed in Supplementary Table 2. The mean age of participants at baseline was 72.53 ± 6.86 years, and their estimated brain age was 75.58 ± 4.79 years. The proportions of participants with a clinical diagnosis of MCI and dementia were 69.2% and 24.3%, respectively.

Identification and characteristics of microbial community types by DMM

Two DMM clusters were identified in the microbiota at the phylum level (Fig. 1 and Supplementary Fig. 1). The cluster 1 group was characterized by six phyla with

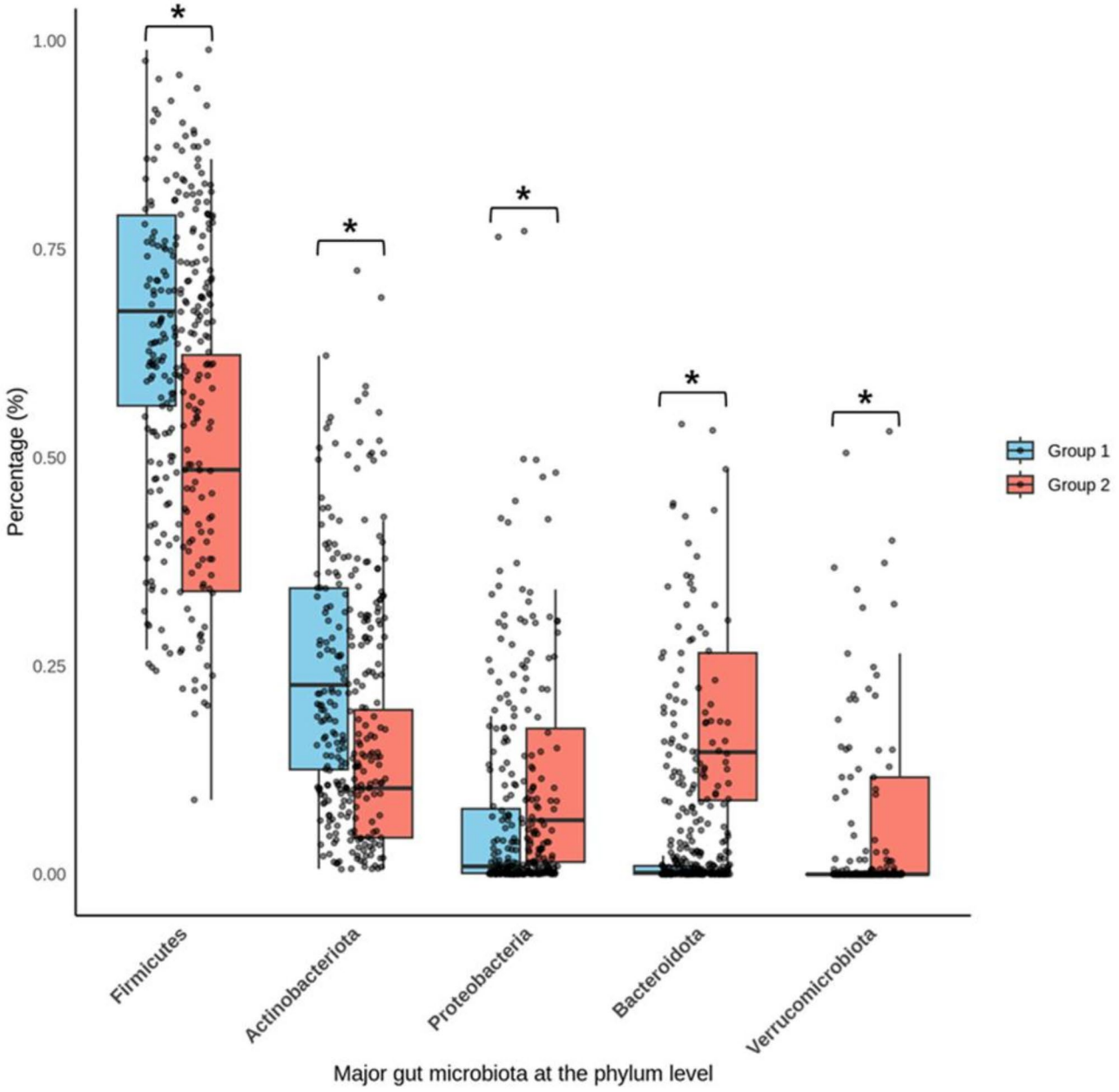

Fig. 1 Dirichlet multinomial mixtures (DMM) clustering using microbiome data

Comparison of major gut microbiota at the phylum level between Group 1 and Group 2. The boxplot represents the relative abundance (%) of six prominent phyla, including Firmicutes, Actinobacteriota, Proteobacteria, Bacteroidota and Verrucomicrobiota. Group 1 is shown in blue, and Group 2 is shown in red. Asterisks indicate statistically significant differences in relative abundance between the two groups ($p < 0.05$)

predominant commensal bacteria: *Firmicutes*, *Actinobacteriota*, *Proteobacteria*, *Bacteroidota*, *Verrucomicrobiota*, and *Euryarchaeota*. Cluster 2 group was characterized by seven phyla with predominant commensal bacteria: *Firmicutes*, *Bacteroidota*, *Actinobacteriota*, *Proteobacteria*, *Verrucomicrobiota*, *Euryarchaeota*, and *Cyanobacteria*. The microbial community was mainly composed of *Firmicutes*, *Bacteroidota*, *Actinobacteriota*, and *Proteobacteria*, which is consistent with the results of previous studies [41]. However, *Bacteroidota*, *Proteobacteria*, *and Verrucomicrobiota* were more abundant in the cluster 2 group than in the cluster 1 group.

The characteristics between the cluster groups did not differ significantly in terms of sex, the CDR score, diagnosis, or clinical assessment (Table 1). However, the cluster 2 group was older ($p = 0.020$), had lower MMSE scores ($p = 0.038$), higher CDR-SB scores ($p = 0.028$), higher brain age_residual ($p = 0.026$), and a higher *Bacteroidetes*/*Firmicutes* ratio ($p < 0.001$) than the cluster 1 group did.

Elastic net predictions

The best model achieved an out-of-bag accuracy of 0.66 predicting groups ($\alpha = 0$, $\lambda = 68.9$, $p < 0.001$). The results for each individual factor are presented in Table 2 and Supplementary Table 3, which shows the pooled *p*-values across the imputations. The cluster 1 group was associated with higher anxiety, lifetime alcohol consumption, and a higher likelihood of thyroid disorders, compared with the cluster 2 group. However, individuals with the cluster 1 group were younger, had lower brain age, lower global amyloid, higher overall cognitive function (working memory, spatial memory, word recall, and overall cognitive function), and higher hematocrit, hemoglobin, HDL, and vitamin B12 levels, compared with individuals with the cluster 2 group. Due to the relatively small number of subjects with Aβ40 data, the Aβ40 and Aβ42/40 variables were excluded from the elastic net model analysis.

Mediational analyses

We explored the mediating roles of brain age between group clusters and cognitive function (measured using MMSE and CDR-SB). For the mediation analysis, we divided the data into two clusters, coding cluster 1 as "0" and cluster 2 as "1." Mediation analyses results are shown in Fig. 2; Table 3, and Table 4. All fit indices met the required standards, indicating a strong overall fit for the model (Supplementary Table 4 in the Additional file 1).

Table 1 Clinical characteristics of participants according to the data-driven cluster groups using microbiome data

	Group 1 (*N* = 191)		Group 2 (*N* = 97)	
	***N*, or mean**	**%, or SD**	***N*, or mean**	**%, or SD**
Age	71.84	7.06	73.84	6.30
Sex (female)	130	68.06	70	72.16
CDR				
0	6	3.14	14	1.03
0.5	158	82.72	74	76.29
1	23	12.04	19	19.59
2	4	2.09	3	3.09
Dx				
SCD	11	5.70	8	8.20
MCI	137	72.00	63	65.30
Dementia	43	22.30	26	26.50
CDR-SB	2.61	2.00	3.02	2.16
MMSE	23.40	4.93	22.86	4.82
MADRS	10.06	12.39	15.70	12.44
BAI	8.53	10.96	9.58	10.89
Lifetime alcohol consumption	17896.61	36091.06	11272.29	36583.95
MNA	20.09	5.48	20.67	4.76
BMI	23.82	3.32	23.94	3.60
Brain age residual	-0.09	0.97	0.19	1.01
Amyloid PET SUVR (***N* = 185, 96**)	0.67	0.16	0.67	0.16
Plasma Aβ42	6.44	3.74	6.40	3.72
Plasma Aβ40 (***N* = 103, 47**)	178.43	63.90	179.19	65.24
Plasma pTau 217	3.00	1.69	3.03	1.70
Bacteroidota/Firmicutes ratio	0.027	0.075	0.473	0.475

Abbreviations: SD, standard deviation; CDR-SB, clinical dementia rating sum of box; MMSE, mini-mental state examination; MADRS, Montgomery-Asberg depression rating scale; BAI, Beck anxiety inventory; BMI, body mass index; MNA, mini nutritional assessment; SCD, subjective cognitive decline; MCI, mild cognitive impairment

Table 2 Factors associated with cluster groups identified by elastic net regression

	fcoeff average	fcoeff SD	*P* vales pooled	Compared to cluster 2, cluster 1 has
Chronological age*	0.001000	0.000018	0.000007	lower age
Brain age_residual*	0.000900	0.000002	0.000035	lower brain age
Amyloid PET SUVR*	0.000800	0.000070	0.001000	lower amyloid
CDR-SB*	0.000600	0.000008	0.019000	better overall cognitive function
BAI*	-0.001000	0.000002	0.000002	higher anxiety
Lifetime Alcohol consumption*	-0.000900	0.000002	0.000011	higher lifetime drinking
MMSE	-0.000321	0.000026	0.565343	
MADRS	-0.000028	0.000025	0.999968	
BMI	0.000136	0.000015	0.980270	
Plasma pTau 217	0.000133	0.000013	0.981084	
Plasma Aβ42	-0.000241	0.000033	0.812965	

Abbreviations: SD, standard deviation; SUVR, standardized uptake value; CDR-SB, clinical dementia rating sum of box; BAI, Beck anxiety inventory; MMSE, mini-mental state examination; MADRS, Montgomery-Asberg depression rating scale; BMI, body mass index. *$P < 0.05$

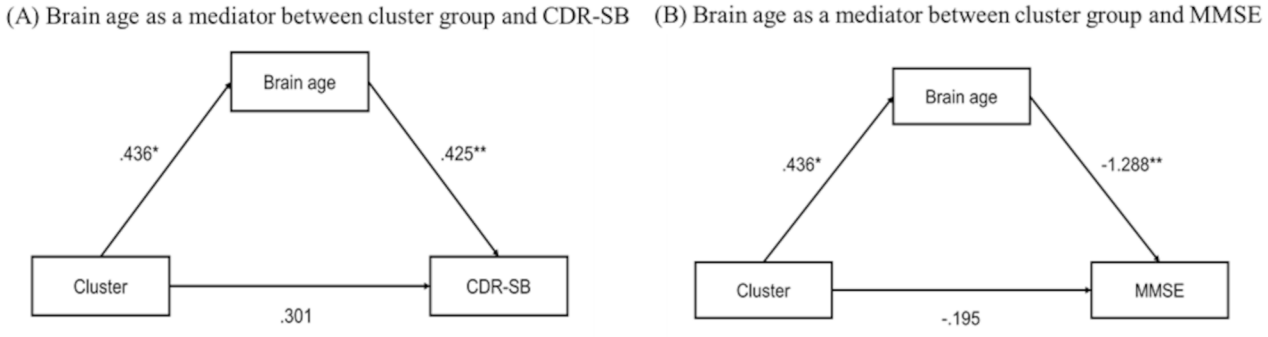

Fig. 2 Mediation analysis among cluster group, brain age, and cognitive function measures
Mediation analysis adjusted for sex, age, and education. Abbreviations: CDR-SB, Clinical Dementia Rating Sum of Box; MMSE, Mini-Mental State Examination; * $p < 0.05$; ** $p < 0.01$

Table 3 presents the estimated regression coefficients for the clusters, brain age, and the MMSE score. In the unadjusted model, individuals in the cluster 2 group were observed to have a greater brain age compared to those in the cluster 1 group (ß =0.283, 95% confidence interval [CI] = 0.044 to 0.523, $p = 0.021$). Furthermore, when controlling for the cluster group, the direct effect of brain age on the MMSE was significant (ß=-1.244, 95% CI=-1.800 to -0.687, $p < 0.001$). Although the direct effect was insignificant, the indirect effect of the cluster group on the MMSE score through brain age was significant (ß=-0.352, 95% CI=-0.690 to -0.015, $p = 0.041$). The mediation analysis, adjusted for sex, age, and education, was consistent with the unadjusted analysis. Specifically, individuals with the cluster 2 group exhibit greater brain age than do those with the cluster 1 group (ß =0.436, 95% confidence interval [CI] = 0.118 to 0.883, $p = 0.024$). When controlling for the cluster group, the direct effect of brain age on the MMSE was significant (ß=-1.288, 95% CI=-1.793 to -0.783, $p < 0.001$). Although the direct effect was insignificant, the indirect effect of the cluster group on the MMSE score through brain age is significant (ß=-0.370, 95% CI=-0.714 to -0.026, $p = 0.035$).

For the CDR-SB score outcome variable in Table 4, individuals with the cluster 2 group exhibited greater brain age than did those with the cluster 1 group (ß=0.283, 95% CI = 0.044 to 0.523, $p = 0.021$) in the unadjusted model. Furthermore, when controlling for the cluster group, the direct effect of brain age on the CDR-SB score was significant (ß=0.425, 95% CI = 0.188 to 0.661, $p < 0.001$). In contrast, both direct and indirect effects of the cluster group on the CDR-SB score through brain age were not significant; however, the indirect effect showed the same tendency as the CDR-SB score outcome at the p value of 0.053 (direct: ß=-0.195, 95% CI=-1.361 to 0.971, $p = 0.743$; indirect: ß=0.120, 95% CI=-0.002 to -0.242, $p = 0.053$, respectively). The results of the mediation analysis, adjusted for sex, age, and education, was consistent with those of the unadjusted analysis. Individuals with the cluster 2 group exhibit greater brain age than do those with the cluster 1 group (ß =0.436, 95% confidence interval [CI] = 0.118 to 0.883, $p = 0.024$). When controlling for the cluster group, the direct effect of brain age

Table 3 Mediation analysis among cluster groups, brain age, and MMSE

	Coeff (ß)	SE	T	*P* value	LLCI	ULCI
Unadjusted analysis						
Outcome: Brain age residual						
Cluster group	0.283	0.122	2.316	0.021*	0.044	0.523
Outcome: MMSE						
Cluster group	-0.195	0.595	-0.328	0.743	-1.361	0.971
Brain age residual	-1.244	0.284	-4.382	< 0.001*	-1.800	-0.687
Indirect effect	-0.352	0.172	-2.047	0.041*	-0.690	-0.015
Direct effect	-0.195	0.595	-0.328	0.743	-1.361	0.971
Total effect	-0.547	0.609	-0.899	0.368	-1.740	0.646
Adjusted analysis						
Outcome: Brain age residual						
Cluster group	0.436	0.193	2.262	0.024*	0.118	0.883
Sex	-0.004	0.127	-0.035	0.972	-0.254	0.245
Age	-0.002	0.009	-0.196	0.845	-0.019	0.015
Education	0.005	0.013	0.360	0.719	-0.020	0.029
Outcome: MMSE						
Cluster group	-0.195	0.545	-0.358	0.720	-1.264	0.873
Brain age residual	-1.288	0.258	-5.000	< 0.001*	-1.793	-0.783
Sex	-0.035	0.557	-0.063	0.950	-1.126	1.056
Age	0.047	0.038	1.245	0.213	-0.027	0.122
Education	0.427	0.055	7.777	< 0.001*	0.319	0.534
Indirect effect	-0.370	0.176	-2.109	0.035*	-0.714	-0.026
Direct effect	-0.195	0.545	-0.358	0.720	-1.264	0.873
Total effect	-0.565	0.563	-1.004	0.315	-1.669	0.538

Abbreviations: MMSE, mini mental status examination; SE, standard error; LLCI, lower limit confidence interval; ULCI, upper limit confidence interval. * $p < 0.05$

Table 4 Mediation analysis among cluster groups, brain age, and CDR-SB

	Coeff (ß)	SE	T	*P* value	LLCI	ULCI
Unadjusted analysis						
Outcome: Brain age residual						
Cluster group	0.283	0.122	2.316	0.021*	0.044	0.523
Outcome: CDR-SB						
Cluster group	0.288	0.252	1.140	0.254	-0.207	0.783
Brain age residual	0.425	0.120	3.524	< 0.001*	0.188	0.661
Indirect effect	0.120	0.062	1.935	0.053	-0.002	-0.242
Direct effect	-0.195	0.595	-0.328	0.743	-1.361	0.971
Total effect	-0.547	0.609	-0.899	0.368	-1.740	0.646
Adjusted analysis						
Outcome: Brain age residual						
Cluster group	0.436	0.193	2.262	0.024*	0.118	0.883
Sex	-0.004	0.127	-0.035	0.972	-0.254	0.245
Age	-0.002	0.009	-0.196	0.845	-0.019	0.015
Education	0.005	0.013	0.360	0.719	-0.020	0.029
Outcome: CDR-SB						
Cluster group	0.301	0.255	1.182	0.237	-0.198	0.800
Brain age residual	0.425	0.120	3.533	< 0.001*	0.189	0.661
Sex	0.108	0.260	0.417	0.677	-0.401	0.618
Age	-0.006	0.018	-0.332	0.740	-0.041	0.029
Education	-0.014	0.026	-0.550	0.583	-0.064	0.036
Indirect effect	0.122	0.063	1.943	0.052	-0.001	0.246
Direct effect	0.301	0.255	1.181	0.238	-0.198	0.801
Total effect	0.423	0.258	1.641	0.101	-0.082	0.929

Abbreviations: CDR-SB, clinical dementia rating sum of box; SE, standard error; LLCI, lower limit confidence interval; ULCI, upper limit confidence interval. * $p < 0.05$

on the CDR-SB was significant (ß=0.425, 95% CI=-0.189 to 0.661, $p < 0.001$). Although the direct effect was insignificant, the indirect effect of the cluster group on the CDR-SB score through brain age shows a trend toward statistical significance (ß=0.122, 95% CI=-0.001 to 0.246, $p = 0.052$).

Mediation model fit was described in the Supplementary Table 4. Overall, the relationship between clusters and brain age consistently showed significant associations in both mediation effect analyses of MMSE and CDR.

Explorative analyses for mediation effects of amyloid SUVR, plasma amyloid Aβ40, Aβ42, Aβ 42/40 and p-tau 217 did not show any significant results (Supplementary Tables 5–14). We also conducted exploratory mediation analyses for anxiety scores, alcohol consumption, and age, which were found to be significant in the elastic net results. Explorative analyses for mediation effects of anxiety scores, alcohol consumption, and agedid not show any significant results (Supplementary Tables 15–17). Although age was not significant for any of the indirect, direct, or total effects, we did observe a significant relationship between age and cluster. This finding supports the appropriateness of including age as an adjustment factor in the main analysis.

Exploratory linear regression analyses

Considering that *Bacteroidota and Verrucomicrobiota* was the primary compositional difference within the clusters, we performed additional analyses to examine the association between brain age and *Bacteroidota and Verrucomicrobiota* at the family level. Additional analyses were conducted to investigate the relationship between microbial strain and brain age within each of the *Bacteroidota and Verrucomicrobiota* families. Among *Bacteroidota* at the family level, Linear regression analysis revealed that *Bacteroidaceae* had a significant association with brain age. ($p = 0.032$) (Supplementary Table 18). On the other hand, no significant results were found in the *Verrucomicrobiota* at the family level (Supplementary Table 19).

Discussion

In this study, we investigated whether brain age impacted the relationship between gut microbiome dysbiosis and cognitive performance in a cohort of older adults with normal cognition, MCI, AD, or SVaD. Our analysis revealed that brain age significantly mediated the relationship between microbiome clusters and cognitive performance, including the MMSE and CDR scores. Moreover, this association for the MMSE score was independent of the chronological age, sex, and education. The complex interplay between the gut microbiome and brain health is shedding new light on our understanding of neurodegenerative diseases. The concept that dysbiosis contributes to accelerated gray matter aging is not only innovative but also poses a substantial challenge to traditional therapeutic approaches. In this discussion, we aim to delve into the mechanisms by which dysbiosis could potentially influence brain aging and discuss the implications for future research and therapy.

Mechanisms of Microbiome Influence on Brain Aging

Recent research has identified key pathways through which the gut microbiota influences the CNS. Dysbiosis triggers neuroinflammation by activating microglia through microbial byproducts [35]. Gut-derived metabolites, such as short-chain fatty acids and Trimethylamine N-oxide, modulate neuroinflammation, oxidative stress, and neuroplasticity [42]. Additionally, vagus nerve serves as a direct link, allowing the gut microbiota to impact stress, anxiety, and memory [43]. These findings emphasize the gut–brain axis's role in neuroinflammatory and neurodegenerative disorders.

Recent studies have demonstrated that gut microbiome dysbiosis significantly impacts the structure and function of gray matter. Yamashiro et al. [8] found that AD and MCI patients exhibited decreased butyrate-producing bacteria, correlating with increased free water in gray matter. Similarly, Lee et al. [9] reported a positive association between gut microbiome diversity and hippocampus volume in older adults with depression. Liang et al. [10] integrated multi-omics data to reveal that *Odoribacter* are linked to hippocampal volume. Additionally, Shi et al. [44] identified associations between gut microbiota composition and cerebral cortical thickness, while Li et al. [45] connected gut microbiome alterations with changes in brain structure and function in schizophrenia patients. Our results also demonstrated that brain age, derived from gray matter volume, is significantly associated with gut microbiome dysbiosis and a mediator in the relationship between microbiome and cognitive function. Collectively, these findings underscore the critical role of gut microbiome composition in influencing gray matter integrity and cognitive health.

Impact of gut microbiome dysbiosis on brain aging and cognitive function

Gut dysbiosis has significant implications for neurodegeneration and brain aging. An imbalance in gut microbiome composition has been linked to an increased production of pro-inflammatory cytokines. These cytokines can cross the blood-brain barrier and contribute to neuroinflammation, which is a recognized factor in accelerating brain aging and the development of neurodegenerative diseases [46]. Regarding the specific composition of dysbiosis, it has been reported that patients with AD exhibit a significant decrease in the proportion

of the phylum *Firmicutes* and a substantial increase in *Proteobacteria* compared to controls [47]. Another study has reported a reduction in *Firmicutes* and an increase in *Bacteroidetes* species in AD [48]. In our clustering results, consistent with those of previous studies, cluster 2 exhibited a lower proportion of *Firmicutes* and higher proportions of *Proteobacteria* and *Bacteroidota* than did cluster 1. Additionally, consistent with previous studies that reported an increased presence of taxa belonging to the phylum *Verrucomicrobiota* in relative abundance analyses of AD patients, the proportion of *Verrucomicrobiota* was significantly higher in cluster 2 [49]. Furthermore, cognitive function, including that measured using MMSE and CDR-SB, was significantly worse and brain age was significantly higher, with significant gut dysbiosis, in the cluster 2 group than in cluster 1 group. In the factor analysis between the groups using elastic nets, brain age showed a significant correlation as well. Additionally, there were differences in specific cognitive function measures, such as spatial memory, naming function, and working memory. Notably, a high HDL level was identified as a factor that contributed less to gut dysbiosis, which aligns with existing explanations regarding neuroinflammation through metabolic inflammation [50].

Alterations in gut microbiota composition, specifically decreased levels of *Firmicutes* and *Actinobacteriota* coupled with increased levels of *Bacteroidota*, *Proteobacteria*, and *Verrucomicrobiota*, have been implicated in the pathogenesis of neurodegenerative diseases such as AD [51]. A reduction in *Firmicutes* and *Actinobacteriota* may lead to decreased production of SCFAs, which are crucial for maintaining the integrity of the blood-brain barrier and modulating neuroinflammation. Conversely, elevated levels of *Bacteroidota* and *Proteobacteria* can result in increased production of lipopolysaccharides (LPS), potent endotoxins that trigger systemic inflammation and promote neuroinflammatory processes contributing to AD pathology. Additionally, changes in *Verrucomicrobiota* populations, such as *Akkermansia muciniphila*, may affect mucin degradation and gut barrier function, further influencing systemic inflammation and neurodegeneration [52]. These microbial shifts can disrupt the gut-brain axis, leading to increased permeability of the gut and blood-brain barriers, facilitating the entry of pro-inflammatory molecules into the central nervous system, and ultimately accelerating neurodegenerative processes [53].

Among the *Bacteroidota* phylum, the *Bacteroidaceae* family has shown a significant association with brain aging. Recent studies have explored the complex relationship between the gut microbiota family *Bacteroidaceae* and neurodegenerative diseases, particularly AD. A two-sample Mendelian randomization analysis utilizing data from the Dutch Microbiome Project and the international MibioGen consortium found that elevated levels of *Bacteroidaceae* are significantly associated with a reduced risk of AD, suggesting a potential protective effect [54]. However, certain members of this family, such as *Bacteroides fragilis*, produce lipopolysaccharides (LPS) that can induce neuroinflammation. LPS from *B. fragilis* has been detected in neuronal nuclei in sporadic AD brains and is known to activate inflammatory pathways in human neuronal-glial cells, leading to the upregulation of pro-inflammatory microRNAs and contributing to neurodegenerative processes [55]. Additionally, research on aging mice has shown that an increased abundance of *Bacteroidaceae* correlates with elevated levels of pro-inflammatory cytokines and brain inflammation, factors implicated in neurodegeneration [56]. These findings highlight the dual role of *Bacteroidaceae* in neurodegenerative diseases, where certain members may offer protective effects while others promote neuroinflammation, underscoring the complexity of their involvement in neurodegenerative processes.

Meanwhile, the factor analysis that identified differences between clusters selected factors such as amyloid SUVR levels and brain age as important factors, but not blood amyloid and tau levels. However, an exploratory mediation analysis revealed that neither blood markers nor amyloid PET SUVR results showed any effect linking microbiome dysbiosis to cognitive decline. These findings suggest that the relationship between gut microbiota and AD pathogenesis remains uncertain. Given the cross-sectional design of this study, further research is needed.

Considering the close relationships among gut dysbiosis, brain age, and cognitive function, our study shed light on the role of gut dysbiosis in cognitive performance, with brain age as a mediating factor. A key finding of our study was that the link between severe gut dysbiosis and poor cognitive performance was partly mediated by brain age.

Clinical and therapeutic implications

Understanding the relationship between microbiome profiles and brain aging may lead to the development of microbial biomarkers that predict the rate of gray matter aging, cognitive decline, and the risk of dementia [47]. Probiotics, prebiotics, and dietary interventions can be explored as potential strategies to modulate the gut microbiome, thereby mitigating neurodegenerative processes and promoting cognitive health in elderly populations [57].

Establishing a causal relationship between the clusters and brain aging could lead to microbiome-based interventions. For example, modifying the diet to improve microbiome dysbiosis may help reduce neuroinflammation and slow gray matter aging. Probiotics and prebiotics

designed to optimize microbiome composition could potentially serve as adjuvant therapies for the management of neurodegenerative diseases.

Limitations

This study has some limitations. First, the current study employed a cross-sectional design, which provides only a snapshot in time. Because the microbiome data are cross-sectional and collected simultaneously with the outcomes, the temporal precedence required for true mediation analysis is not met, making it impossible to infer causal relationships. Additionally, since most participants in the study have cognitive impairment, the observed alterations in microbiome composition are likely influenced by lifestyle and environmental changes associated with dementia symptoms. Therefore, longitudinal studies are essential to clarify the causal links between microbiome changes and brain aging. Such studies would offer insights into the dynamic interactions between the gut microbiome and brain health over extended periods, enhancing our understanding of the long-term effects. Second, this study was conducted exclusively with South Korean participants. The gut microbiome may be influenced by genetic and social environmental factors. Further studies incorporating greater diversity and considering geographic, cultural, and genetic ancestry differences are needed to better contextualize these results. Third, we conducted microbiome analysis limited to the phylum level. Previous studies have reported that using species-level data can better distinguish between normal and abnormal conditions compared to the phylum level [58]. However, other research has also found group differences using phylum-level information. Considering the complexity involved in integrating various types of data for analysis, we performed our study at the phylum level, but species-level analysis will be necessary in future research [59]. Fourth, we focused solely on an older population with varying degrees of cognitive function. As the results may vary by age group and cognitive function level, further studies involving different age groups and disease populations are required.

Conclusions

We found that brain age mediated the relationship between cluster-related gut microbiome dysbiosis and cognitive performance. Our results suggest that the relationship between increased gut microbiome dysbiosis and worsened cognitive performance may be partially mediated by brain age. The implications of these findings could pave the way for novel interventions targeting the gut microbiome to mitigate age-related cognitive decline and improve the quality of life in older adults.

Abbreviations

MMSE	Mini-Mental State Examination
CDR-SB	Clinical Dementia Rating Sum of Box
AD	Alzheimer's disease
BICWALZS	Biobank Innovations for chronic Cerebrovascular disease With ALZheimer's disease Study
SCD	Subjective cognitive decline
MCI	Mild cognitive impairment
SVaD	Subcortical vascular dementia
CDR	Clinical Dementia Rating
WMH	White matter hyperintensity
HbA1c	Glycated hemoglobin
APOE	Apolipoprotein E
DMM	Dirichlet multinomial mixtures
HDL	High-density lipoprotein
CNS	Central nervous system

Supplementary Information

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Additional file 1

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Author contributions

Conceptualization/Funding acquisition: SJS, CHH, and HWR; Data generation: SJS, CHH, HWR, TP, PF, BB, BP, NK, JWC, JYC, YKK, TSS, CUK, COK, SYY; Data Curation: SJS, HWR; Formal Analysis: DYL, HTK, SJS; Original Draft: SJS, DYL, HTK; and Reviewing and Editing: ML, AK, HA, CA, EJ, YHC, SH, YJN.

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Data availability

Data is provided within the manuscript.

Declarations

Ethics approval and consent to participate

BICWALZS was registered in the Korean National Clinical Trial Registry (KCT0003391|| Registration Date: 2018/07/04|| http://cris.nih.go.kr/cris/en/use_guide/cris_introduce.jsp). The research protocol was approved by the Institutional Review Boards of Ajou University Hospital (AJOUIRB-SUR-2021-038) and conducted in accordance with the current version of the Declaration of Helsinki. Written informed consent was obtained from all the participants and caregivers.

Consent for publication

Not applicable.

Competing interests
The authors declare no competing interests.

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