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Original Article

A retrospective study on blood microbiota as a marker for cognitive decline: implications for detecting Alzheimer's disease and amnestic mild cognitive impairment in Republic of Korea

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ABSTRACT

Objectives: This study aimed to investigate the relationship between blood microbiota, specifically bacterial DNA, and cognitive decline in individuals with subjective cognitive decline (SCD) and amnestic mild cognitive impairment (aMCI). The objective was to identify potential microbial signatures that could serve as biomarkers for cognitive deterioration.

Methods: Forty-seven participants were recruited, including 13 with aMCI, 20 with SCD, and 14 normal cognition (NC). Blood samples were collected, and microbial DNA was analyzed using 16S rRNA sequencing on the Illumina MiSeq platform. Bioinformatics analyses—including α- and β-diversity measures and differential abundance testing (using edgeR)—were employed to assess microbial diversity and differences in bacterial composition among groups. Logistic regression models were used to evaluate the predictive impact of the microbiota on cognitive decline.

Results: Microbial diversity differed significantly between groups, with NC exhibiting the highest α-diversity. Both the aMCI and SCD groups showed reduced diversity. Taxa such as Bacteroidia, Alphaproteobacteria, and Clostridia were significantly decreased in the aMCI group compared to NC ($p < 0.05$). In contrast, Gammaproteobacteria increased significantly in the aMCI group compared to both NC and SCD, indicating progressive microbial changes from SCD to aMCI. No significant differences were found between the NC and SCD groups.

Conclusion: Distinct bacterial taxa—particularly the increase in Gammaproteobacteria along with decreases in Bacteroidia, Alphaproteobacteria, and Clostridia—are associated with the progression of cognitive decline. These findings suggest that blood microbiota could serve as potential biomarkers for the early detection of aMCI. However, the small sample size and the lack of control for confounding factors such as diet and medication limit the findings. Larger studies are needed to validate these results and further explore the role of microbiota in neurodegeneration.

Keywords: Brain-gut axis; Cognitive dysfunction; Microbiota

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Introduction

Based on findings from the World Alzheimer Report, it is estimated that approximately 47 million individuals worldwide are currently affected by dementia. Projections indicate that this number may increase to around 131 million by 2050 [1,2]. Alzheimer's disease (AD), the most prevalent form of dementia, is characterized by the gradual degeneration of neurons and cognitive function [3]. In the Republic of Korea, AD is recognized as a primary contributor to dementia, as supported by scholarly research [4]. AD typically progresses through 3 distinct stages: the preclinical stage, amnestic mild cognitive impairment (aMCI), and dementia. aMCI, the most common form of mild cognitive impairment, has a high likelihood of progressing to AD [5]. Decades of research have consistently demonstrated that the accumulation of amyloid beta (Aβ) peptide is closely associated with the onset of AD. Furthermore, subsequent pathological changes—such as the abnormal phosphorylation of tau protein—contribute to AD progression by promoting inflammation-induced neurodegeneration [6]. Nonetheless, the etiology of AD remains uncertain [7].

Recent studies indicate that gut dysbiosis, which influences brain immune homeostasis via the microbiota–gut-brain axis, may play a significant role in the etiology of neurodegenerative disorders [8]. Increased gut permeability may serve as the primary source of bacterial DNA in the bloodstream, in addition to contributions from the skin, oral cavity, and reproductive and respiratory tracts. Although the concentration of bacterial DNA in blood is not linked to sepsis, its elevation may precipitate various brain-related pathologies, including Alzheimer's and Parkinson's diseases [9]. Evidence indicates that bacterial DNA can alter the blood-brain barrier (BBB), hyperactivate the innate immune system, and provoke neuroinflammation—ultimately leading to cognitive impairment [10–12].

This study seeks to examine the correlation between bacterial 16S rRNA in blood—measured using next-generation sequencing technology—and cognitive deterioration in individuals with SCD and aMCI [13].

HIGHLIGHTS

- Significant shifts in blood microbiota were observed between normal cognition and individuals with amnestic mild cognitive impairment (aMCI), including decreases in Bacteroidia, Alphaproteobacteria, and Clostridia.
- A significant increase in Gammaproteobacteria was identified in the aMCI group, suggesting its potential as a biomarker for cognitive decline.
- The findings emphasize the potential of blood microbiota in the early detection and improved understanding of Alzheimer's disease and cognitive impairments.

Materials and Methods

Clinical Demographics

The objectives of this study were explained to all participants or their legally authorized caregivers, and informed consent was obtained from all subjects. The research received approval from the ethical committee of Soonchunhyang University Bucheon Hospital, Bucheon, Republic of Korea. A total of 47 participants were recruited, including 13 individuals with aMCI, 20 with SCD, and 14 normal cognition (NC).

The participants' ages ranged from 47 to 85 years, and each had at least 6 years of formal education. All participants underwent a comprehensive review of their medical history, along with neurological and cognitive assessments, including the administration of the Mini-Mental State Examination (MMSE). The assessment tools employed in this study included the MMSE, the clinical dementia rating (CDR), and *APOE* testing via whole exome sequencing (Table 1).

Sample Collection and DNA Extraction

Participants were instructed to provide a complete blood sample using sterile collection containers. The samples were promptly transported to the laboratory at 4 °C. Because several chemicals used in the real-time polymerase chain reaction (PCR) and sequencing pipeline contain significant quantities of bacterial DNA, there exists a potential for erroneous identification of contaminant DNA in the samples [14]. DNA extraction was conducted with meticulous care to mitigate the risk of cross-contamination or researcher-induced contamination. All extractions were performed by a single individual over 3 consecutive days. DNA was extracted from peripheral blood leukocytes using a conventional phenol/chloroform extraction method as previously described [15]. Following ethanol precipitation, the DNA was resuspended in double-distilled water (ddH_2O) and stored at −80 °C until use. All extractions were conducted within a Class II biological safety cabinet. Genomic DNA concentration in each blood sample was quantified using a NanoDrop 2000 spectrophotometer (Thermo Scientific).

16S rRNA Amplicon Sequencing

The V3–V4 regions of the 16S rDNA were amplified using universal primers (341F and 806R) linked to indices and sequencing adapters. PCR amplification was performed

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Table 1. Demographic characteristics of study participants

Patient ID	Diagnosis	Age (y)	Sex	MMSE	CDR score
PRM12_0003	NC	66	Male	28	0.5
PRM12_0004	NC	82	Male	27	0.5
PRM12_0008	NC	60	Female	29	0.5
PRM12_0010	NC	66	Female	29	0
PRM12_0012	NC	82	Female	24	0.5
PRM12_0013	NC	50	Male	30	0
PRM12_0016	NC	56	Male	28	0.5
PRM12_0019	NC	64	Female	26	0
PRM12_0022	NC	67	Male	24	0
PRM12_0024	NC	77	Female	27	0.5
PRM12_0094	SCD	66	Female	27	0
PRM12_0117	aMCI	61	Female	27	0
PRM12_0127	aMCI	76	Male	24	0.5
PRM12_0136	aMCI	69	Male	24	0
PRM12_0138	aMCI	82	Female	24	0.5
PRM12_0177	SCD	86	Male	27	0.5
PRM12_0178	SCD	62	Female	24	0.5
PRM12_0189	SCD	59	Male	29	0.5
PRM12_0194	NC	82	Male	27	0.5
PRM12_0195	SCD	80	Female	28	0
PRM12_0197	aMCI	87	Male	20	0.5
PRM12_0200	SCD	79	Male	28	0.5
PRM12_0202	SCD	73	Female	26	0.5
PRM12_0203	NC	60	Female	29	0.5
PRM12_0205	NC	66	Female	29	0
PRM12_0207	SCD	51	Female	29	0.5
PRM12_0209	SCD	71	Female	24	0.5
PRM12_0210	aMCI	76	Female	25	0.5
PRM12_0213	SCD	72	Female	28	0.5
PRM12_0214	SCD	68	Female	26	0.5
PRM12_0215	SCD	50	Female	27	0.5
PRM12_0216	SCD	76	Female	23	0.5
PRM12_0217	SCD	78	Female	25	0.5
PRM12_0221	aMCI	79	Male	26	0.5
PRM12_0222	SCD	78	Female	26	0.5
PRM12_0223	SCD	70	Female	25	0.5
PRM12_0226	SCD	60	Female	24	0.5
PRM12_0228	SCD	68	Female	27	0.5
PRM12_0231	SCD	56	Female	24	0.5
PRM12_0233	SCD	55	Female	26	0.5
PRM12_0240	aMCI	74	Male	24	0.5
PRM12_0245	NC	82	Female	24	0.5
PRM12_0263	aMCI	72	Female	19	0.5
PRM12_0267	aMCI	66	Female	25	0.5
PRM12_0271	aMCI	78	Female	26	0.5
PRM51_0019	aMCI	73	Female	20	0.5
PRM66_0018	aMCI	71	Female	26	0.5

For each participant, the following variables are recorded: ID, diagnosis, age, sex, MMSE score, and CDR score.
MMSE, Mini-Mental State Examination; CDR, clinical dementia rating; NC, normal control; SCD, subjective cognitive decline; aMCI, amnestic mild cognitive impairment.

in 20-μL reactions containing 15× polymerase mix (Life Technologies), 20 μM of both forward and reverse primers, and 30 ng of template DNA. The resulting libraries were sequenced on an Illumina MiSeq platform, generating paired-end reads of 250 base pairs in length.

Bioinformatic Analysis

The reads were clustered into operational taxonomic units (OTUs) based on a 97% sequence similarity threshold. Taxonomic classification of the OTUs was determined using Quantitative Insights Into Microbial Ecology and compared against the Greengenes database version 13.8 [16]. The downstream data analysis was performed using the EasyAmplicon v1.21 Pipeline (https://github.com/YongxinLiu/EasyAmplicon) [17]. Bacterial diversity was assessed using α-diversity metrics such as Shannon's index, Simpson index, Chao1, and ACE, along with β-diversity analysis via principal coordinates analysis (PCoA) [18]. Analysis of variance (ANOVA) was conducted to compare α-diversity across groups, and PERMANOVA was employed to assess the clustering of microbial communities via PCoA.

Statistical analyses were performed using STAMP software (https://beikolab.cs.dal.ca/software/STAMP) [19], and functional differences in orthologs between groups were evaluated with 1-way ANOVA followed by Tukey–Kramer multiple comparisons using IBM SPSS ver. 27.0 (IBM Corp.). To evaluate the potential predictive impact among NC, SCD, and aMCI groups, multivariable logistic regression models were constructed using a stepwise approach (likelihood backward) based on the relative abundance of the blood microbiota. Inclusion and exclusion thresholds were set at 0.05 and 0.01, respectively.

Statistical significance was determined using the Student t-test or Mann-Whitney U-test for comparisons between 2 groups, and 1-way ANOVA or the Kruskal-Wallis test for comparisons among more than 2 groups. The Pearson chi-square test, followed by a *post-hoc* test, was used to compare categorical variables. Correlations between variables were computed using edgeR [20].

Ethics Approval

This study protocol was reviewed and approved by the institutional review board (IRB) of Soonchunhyang University Bucheon Hospital (SCHBC-2020-03-016-002). All participants signed an informed consent form approved by the IRB prior to participation.

Results

Clinical Demographics

The demographic and clinical characteristics of the participants in the 3 groups are presented in Table 1. The results indicate no statistically significant differences among the NC, aMCI, and SCD groups in terms of age, sex, *APOE* ε4 carrier status, MMSE scores, CDR scores, or CDR-sum of boxes (SB) (Table 1). It is important to note, however, that substantial disparities existed among the groups with respect to educational backgrounds and MMSE scores [21]. Participants diagnosed with aMCI were identified using previously established criteria [22]. These criteria included a confirmed memory complaint (validated by an informant), the ability to perform daily activities without significant impairment, MMSE scores ranging from 24 to 30, and a CDR score of 0.5. Healthy controls were selected to ensure proportional representation of sex and age within the community. Most of these controls were spouses of patients who had cohabited for at least 20 years and shared similar dietary patterns. These individuals exhibited MMSE scores between 24 and 30, CDR scores of 0, and no notable memory-related concerns.

Comparison of Clinical and Demographic Variables

A comprehensive comparison of clinical and demographic variables was conducted between the NC, SCD, and aMCI groups, with key differences observed in cognitive function, genetic risk factors, and certain clinical scores.

Regarding age, no significant differences were noted between the NC and SCD groups (mean difference = 0.671, $p = 0.842$), nor between the NC and aMCI groups (mean difference = −5.582, $p = 0.142$). However, a trend towards a younger age in the aMCI group compared to the SCD group was observed (mean difference = −6.254, $p = 0.077$), although this did not reach statistical significance. This suggests that age may not be a primary distinguishing factor between the NC and SCD groups, but it might be relevant in differentiating aMCI from SCD.

In the sex comparison, no significant differences were found between the NC and aMCI groups (mean difference = −0.044, $p = 0.803$), or between the SCD and aMCI groups (mean difference = −0.235, $p = 0.157$), suggesting that sex does not significantly influence the classification of these groups. However, a near-significant trend was observed between the NC and SCD groups (mean difference = −0.279, $p = 0.088$), indicating potential but not definitive sex-related differences.

The MMSE, a widely used tool for assessing cognitive function, revealed significant differences in cognitive performance between the groups. Specifically, the aMCI group showed significantly lower MMSE scores than the NC group (mean difference = 3.368, $p < 0.001$), while the SCD group also had significantly lower MMSE scores compared to the aMCI group (mean difference = 2.304, $p = 0.004$). However, no significant difference was found between the NC and SCD groups (mean difference = 1.064, $p = 0.157$). These findings highlight that MMSE scores are sensitive to cognitive changes, particularly between the NC and aMCI groups, with the SCD group showing intermediate scores.

In the CDR score, no significant differences were observed between the NC and SCD groups (mean difference = −0.129, $p = 0.067$), or between the NC and aMCI groups (mean difference = −0.102, $p = 0.184$). Similarly, no significant difference was found between the SCD and aMCI groups (mean difference = 0.027, $p = 0.702$). These results suggest that CDR scores alone may not be sufficient for distinguishing between these groups, as they reflect more subtle cognitive impairments.

The CDR-SB score, which provides a more detailed assessment of cognitive decline, revealed significant differences between the NC and SCD groups (mean difference = −0.621, $p = 0.035$) and between the NC and aMCI groups (mean difference = −0.725, $p = 0.026$). However, no significant difference was found between the SCD and aMCI groups (mean difference = −0.104, $p = 0.720$). These findings suggest that the CDR-SB may be more sensitive than the CDR score in detecting subtle cognitive changes, particularly in the early stages of cognitive decline.

Finally, the APOE genotype comparison showed significant differences between the NC and aMCI groups (mean difference = −0.308, $p = 0.034$) and between the SCD and aMCI groups (mean difference = −0.358, $p = 0.009$), indicating a stronger association of the APOE ε4 allele with aMCI. This supports previous research linking the APOE ε4 allele with increased risk of Alzheimer's disease and other forms of cognitive impairment. No significant difference in the APOE genotype was found between the NC and SCD groups (mean difference = 0.05, $p = 0.694$), suggesting that the APOE genotype is more relevant for distinguishing between aMCI and the other two groups.

These results collectively highlight the differences in clinical and demographic variables across the NC, SCD, and aMCI groups, with significant findings related to cognitive function (MMSE, CDR-SB) and genetic risk factors (APOE genotype). These variables could serve as important markers for distinguishing between different stages of cognitive decline, particularly in identifying individuals at risk for progressing from SCD to aMCI (Table 2).

Landscape of the Microbiome in Blood Sample

Alpha diversity analyses of the blood microbiota revealed significant differences in microbial richness and evenness

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Table 2. Comparison of clinical and demographic variables

	Comparison	Mean difference	SE	95% CI	*p*
Age	NC vs. SCD	0.671	3.346	−6.162 to 7.504	0.842
	NC vs. aMCI	−5.582	3.698	−13.134 to 1.970	0.142
	SCD vs. aMCI	−6.254	3.421	−13.241 to 0.733	0.077
Sex	NC vs. SCD	−0.279	0.158	−0.602 to 0.044	0.088
	NC vs. aMCI	−0.044	0.175	−0.401 to 0.313	0.803
	SCD vs. aMCI	−0.235	0.162	−0.566 to 0.096	0.157
MMSE	NC vs. SCD	1.064	0.733	−0.433 to 2.561	0.157
	NC vs. aMCI	3.368	0.81	1.714 to 5.022	< 0.001
	SCD vs. aMCI	2.304	0.749	0.774 to 3.834	0.004
CDR score	NC vs. SCD	−0.129	0.068	−0.268 to 0.010	0.067
	NC vs. aMCI	−0.102	0.075	−0.255 to 0.051	0.184
	SCD vs. aMCI	0.027	0.07	−0.116 to 0.170	0.702
CDR-SB	NC vs. SCD	−0.621	0.281	−1.195 to −0.047	0.035
	NC vs. aMCI	−0.725	0.31	−1.358 to −0.092	0.026
	SCD vs. aMCI	−0.104	0.287	−0.690 to 0.482	0.720
APOE genotype	NC vs. SCD	0.05	0.126	−0.270 to 0.307	0.694
	NC vs. aMCI	−0.308	0.139	−0.592 to −0.024	0.034
	SCD vs. aMCI	−0.358	0.128	−0.619 to −0.097	0.009

The results of *post-hoc* comparisons between the NC, SCD, and aMCI groups for several key clinical and demographic variables, including age, gender, MMSE score, CDR score, CDR-SB score, and *APOE* genotype. The table includes mean differences between each pair of groups, the standard error of the mean difference, *p*-values, and 95% confidence intervals.

SE, standard error; CI, confidence interval; NC, normal cognition; SCD, subjective cognitive decline; aMCI, amnestic mild cognitive impairment; MMSE, Mini-Mental State Examination; CDR, clinical dementia rating; CDR-SB, clinical dementia rating sum of boxes.

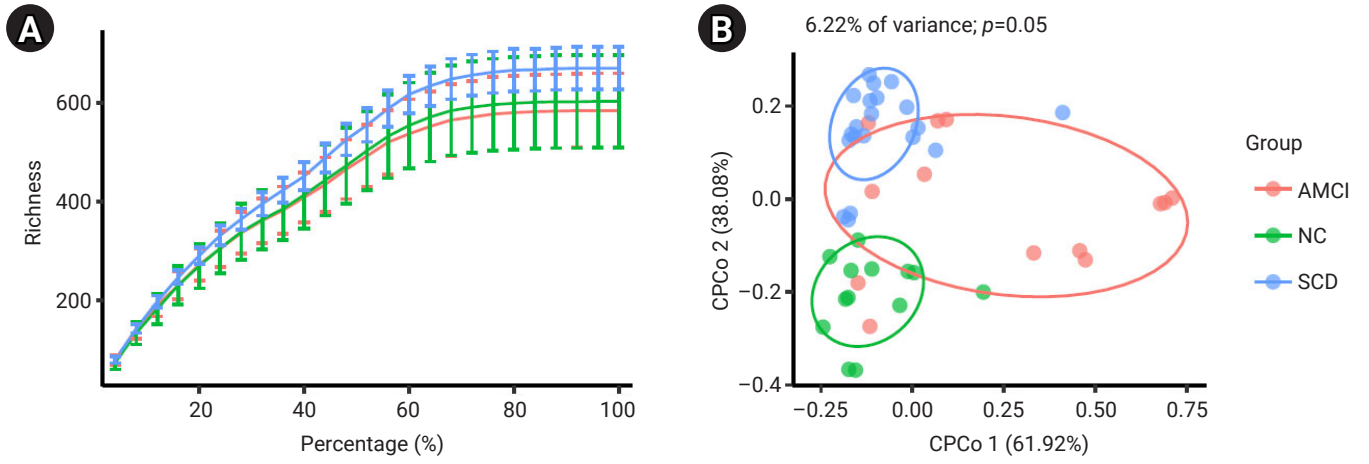

Figure 1. Alpha and beta diversity analysis. (A) Alpha rarefaction curve; richness increases with sequencing depth, approaching saturation, indicating adequate sampling. Variations in curve trajectories suggest differences in biodiversity among the groups (amnestic mild cognitive impairment [aMCI], normal cognition [NC], and subjective cognitive decline [SCD]). (B) Beta diversity (CPCoA): CPCoA shows differences in community composition between groups. Ellipse overlaps indicate similarities, while separations highlight microbial divergence, with 6.22% of variance explained ($p = 0.05$).

among the NC, aMCI, and SCD groups. The Shannon index and Chao1 index were used to assess diversity (Figure 1A). NC exhibited the highest alpha diversity, while both the aMCI and SCD groups demonstrated significantly lower diversity. One-way ANOVA results indicated significant differences in microbial diversity and richness across the 3 groups, and *post-hoc* Tukey honest significant difference tests confirmed pairwise differences, with NC showing the highest diversity, followed by SCD and aMCI. This gradient of reduced microbial diversity may be linked to disease progression

Table 3. Microbial diversity and richness metrics across groups

Metric	*n*	Mean ± SD	ANOVA (*p*)	Significant difference (Tukey HSD)
Chao1			< 0.001	NC > SCD, NC > aMCI, SCD > aMCI
0	14	832.5 ± 24.4		
1	20	607.5 ± 13.33		
2	13	416.92 ± 20.97		
Richness			< 0.001	NC > SCD, NC > aMCI, SCD > aMCI
0	14	1,460.71 ± 21.56		
1	20	1,031.5 ± 31.5		
2	13	660.31 ± 39.4		
Simpson			< 0.001	NC > SCD, NC > aMCI, SCD > aMCI
0	14	0.5526 ± 0.0372		
1	20	0.4554 ± 0.0141		
2	13	0.3064 ± 0.024		

The table summarizes microbial diversity and richness indices across 3 groups (NC, SCD, aMCI). Significant differences ($p < 0.05$) were found between groups for all indices based on ANOVA. Tukey honest significant difference *post-hoc* tests confirmed pairwise differences, indicating that group 0 consistently had the highest diversity, followed by group 1, and group 2 with the lowest (0 = NC, 1 = SCD, 2 = aMCI).
SD, standard deviation; ANOVA, analysis of variance; HSD, honest significant difference; NC, normal cognition; SCD, subjective cognitive decline; aMCI, amnestic mild cognitive impairment.

(Table 3). The decrease in microbial diversity observed in the aMCI and SCD groups may reflect early alterations in blood microbiota composition associated with cognitive decline. These findings are consistent with previous studies suggesting that reduced microbial diversity correlates with neurodegenerative diseases.

These results highlight the potential of blood microbiome composition as a biomarker for distinguishing between healthy aging and the early stages of cognitive decline, such as aMCI and SCD. The clear separation observed in beta diversity analysis underscores the possibility of identifying microbial signatures indicative of neurodegenerative changes. Further studies are needed to explore the mechanisms driving these microbiome shifts and their potential role in cognitive impairment. Both analytical methods yielded consistent results, underscoring the robustness of the findings. This progressive decline in microbial diversity suggests that gut dysbiosis may contribute to cognitive impairment and that blood microbiome analysis could serve as a valuable tool for identifying biomarkers and elucidating the mechanisms underlying neurodegeneration.

Differential Abundance of Bacterial Taxonomy

A differential abundance analysis of bacterial taxa between the NC and aMCI groups was conducted using edgeR. The results revealed several significant shifts in bacterial composition. Specifically, Bacteroidia, Alphaproteobacteria, and Clostridia were significantly decreased in the aMCI group compared to NC, whereas Gammaproteobacteria showed a significant increase in abundance in the aMCI group (Figure 2). These findings suggest that specific bacterial taxa may be associated with the progression from normal cognitive function to aMCI, with alterations in microbial communities potentially contributing to the underlying pathophysiology of the condition.

Additionally, the analysis revealed a significant increase in the abundance of Gammaproteobacteria in the aMCI group compared to the SCD group ($p < 0.05$) (Figure 3A). However, no significant differences in bacterial abundance were observed between the NC and SCD groups (Figure 3B). These findings suggest that while microbial changes are evident between NC and aMCI, there are no major shifts between NC and SCD. Gammaproteobacteria may serve as a key marker in the transition from SCD to aMCI (Figure 3C). These observations underscore the importance of further exploring specific bacterial taxa as potential biomarkers for the early detection and monitoring of cognitive decline.

Discussion

This study represents one of the initial attempts to characterize the blood microbiome in older Korean patients exhibiting symptoms of aMCI. It specifically aimed to compare microbial composition across clinical phases, including aMCI, SCD, and NC. Our findings revealed significant differences in the microbiome composition of patients in the early stages of cognitive decline compared to NC. These differences underscore the potential role of blood microbiota in the pathophysiology of cognitive decline and neurodegenerative diseases.

Our results demonstrated a decreased abundance of Bacteroidia in aMCI and AD patients. This class of bacteria is

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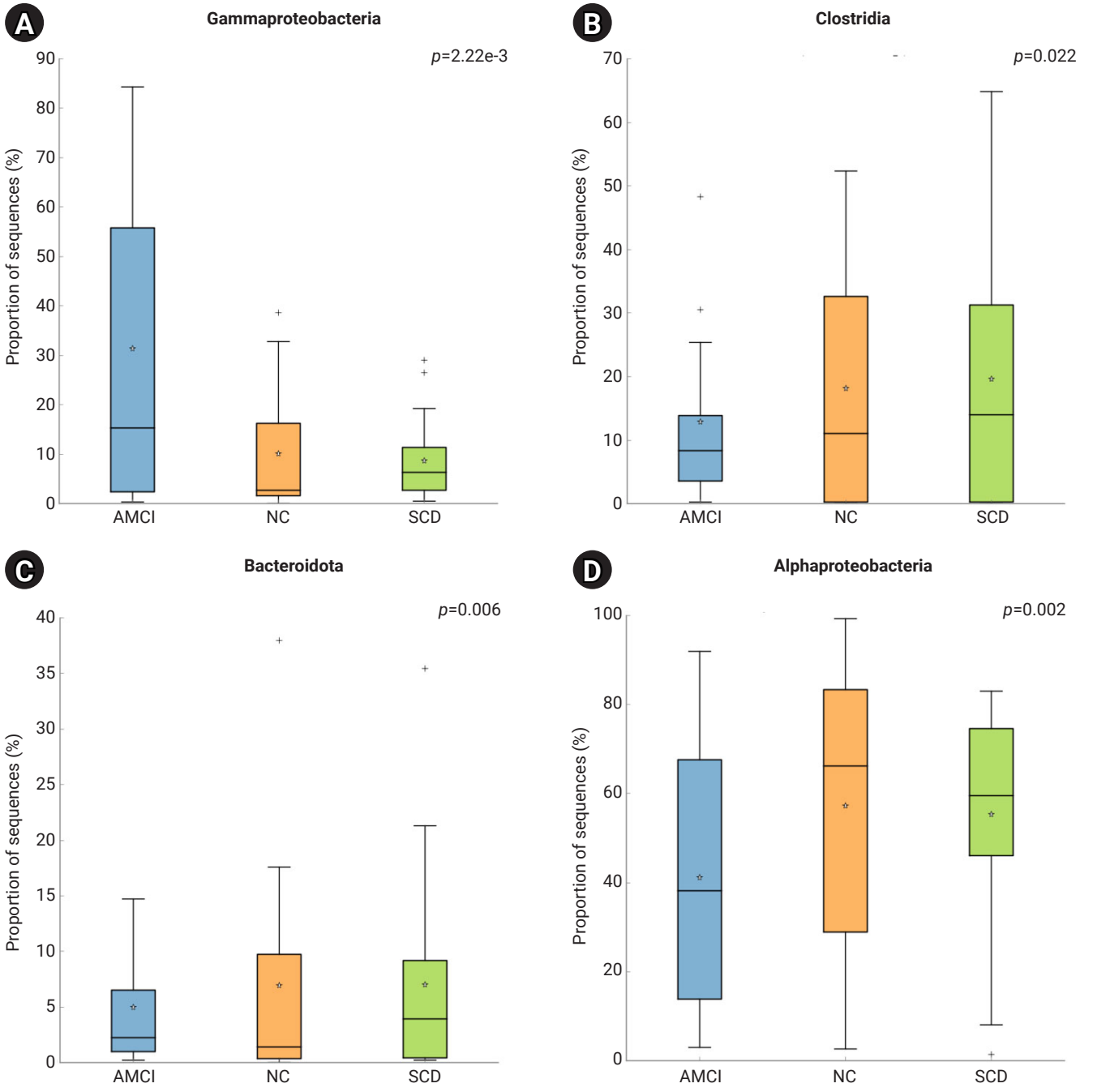

Figure 2. Relative abundances of bacterial taxa in the amnestic mild cognitive impairment (aMCI), normal cognition (NC), and subjective cognitive decline (SCD) groups based on STAMP analysis of blood 16S rRNA sequencing. (A) Gammaproteobacteria: significantly higher in aMCI ($p < 0.002$). (B) Clostridia: higher in NC and SCD than in aMCI ($p = 0.022$). (C) Bacteroidota: more abundant in NC ($p = 0.006$). (D) Alphaproteobacteria: highest in NC, followed by SCD and aMCI ($p = 0.002$). Box plots represent median (line inside the box), mean (◇), interquartile range (IQR; box edges), and whiskers indicating 1.5×IQR. Plus sign (+) indicates an outlier.

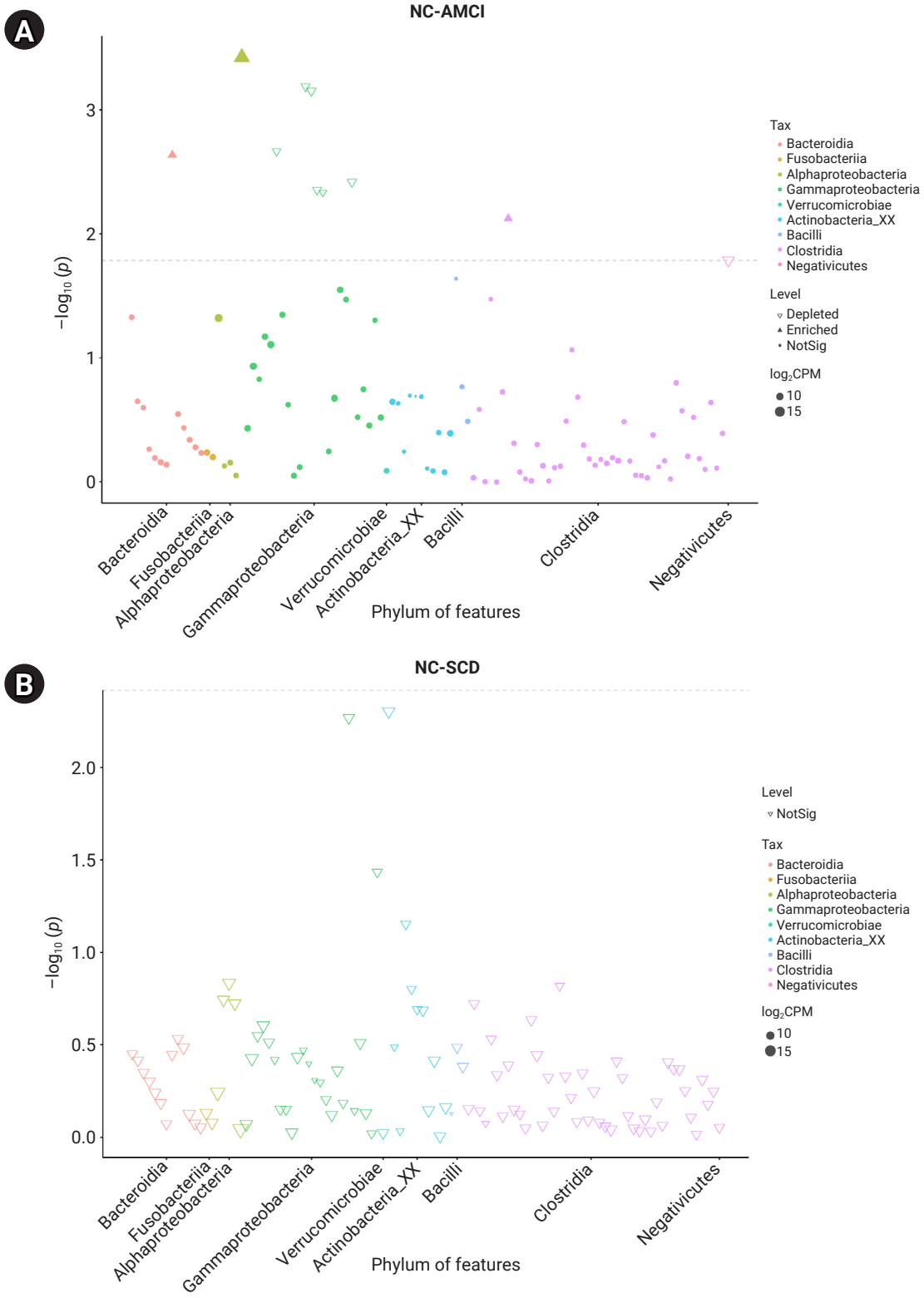

Figure 3. (Continued on next page.)

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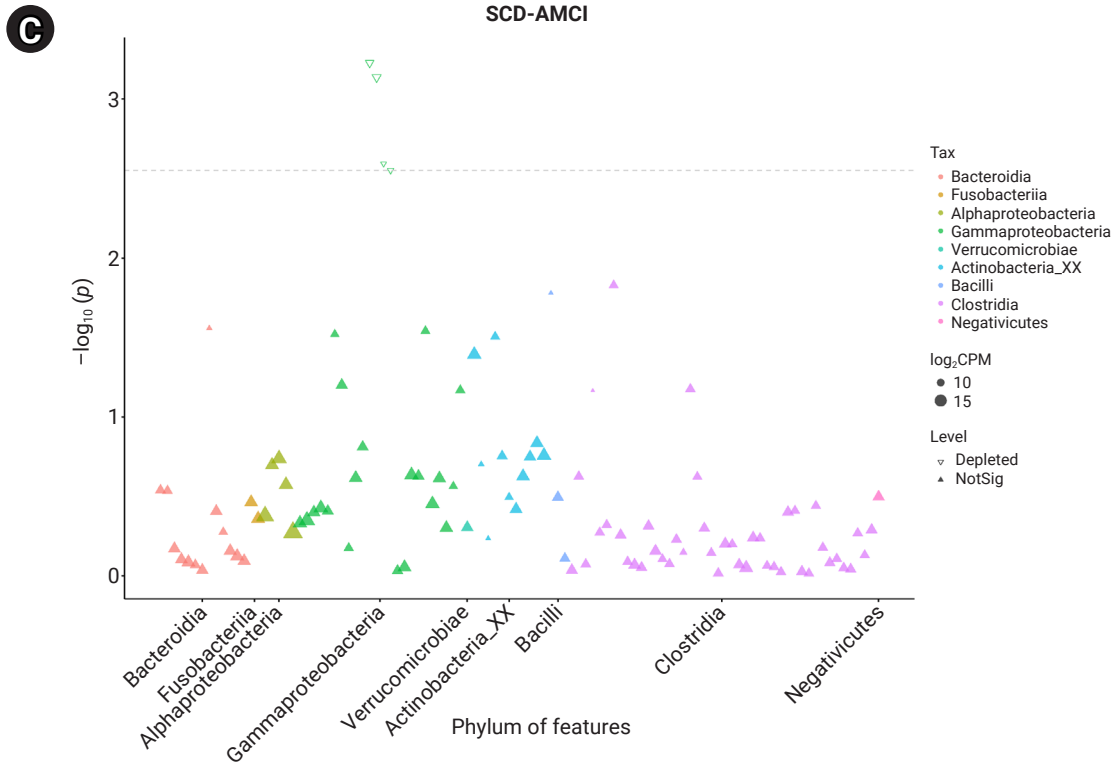

Figure 3. Manhattan plot of bacterial abundance associations. (A) Normal cognition (NC) vs. amnestic mild cognitive impairment (aMCI): increased Alphaproteobacteria (beneficial symbionts) and decreased Gammaproteobacteria (opportunistic pathogens) in aMCI. (B) NC vs. subjective cognitive decline (SCD): no significant bacterial associations ($p > 0.05$), indicating minimal microbiome differences. (C) SCD vs. aMCI: significant decrease in Gammaproteobacteria in aMCI, reflecting microbiome changes with cognitive decline. The x-axis represents taxa (log2CPM), and the y-axis shows −log10 p-values. Significant associations are highlighted above the threshold.

associated with inflammatory processes in the gut, systemic inflammation, and disruptions to the gut-brain axis, which may influence the progression of dementia [23]. Similarly, Alphaproteobacteria—which promote cytokine production and exacerbate neuroinflammation—were significantly decreased in aMCI and AD patients [24]. Interestingly, Clostridia, known for their role in producing short-chain fatty acids (SCFAs), exhibited a complex influence; while SCFAs possess neuroprotective properties, a reduction in Clostridia populations could lead to increased gut permeability and systemic inflammation [25]. Conversely, we observed a significant increase in Gammaproteobacteria, bacteria linked to dysregulation of gut homeostasis, suggesting a shift toward a dysbiotic state in aMCI and AD patients [26].

These findings emphasize the critical role of gut and blood microbiota in maintaining the integrity of the gut-brain axis. Dysbiosis may promote chronic neuroinflammation, disrupt the BBB, and impair the production of neuroactive metabolites, all of which contribute to cognitive decline [27,28]. The identification of microbial signatures specific to aMCI and AD may inform the development of novel diagnostic tools and therapeutic strategies targeting the gut-brain axis.

Conclusion

This research highlights the potential of blood microbiota as biomarkers and therapeutic targets for AD and aMCI. By identifying specific microbial signatures associated with these conditions, the study lays the foundation for developing novel strategies to prevent, diagnose, and treat neurodegenerative diseases. Addressing the limitations discussed and expanding future research to include dietary, medication, and genetic influences will further enhance our understanding of the gut-brain axis and its role in cognitive health.

Diet and Medication as Confounders

This study did not account for the influence of diet and medication on microbiome composition and cognitive decline. Both factors significantly shape microbial populations and could confound the associations observed in this research [29]. Future studies should employ statistical methods to adjust for these variables or directly investigate

their impact on the microbiome and cognitive function.

Technical Limitations

While 16S rRNA sequencing provided valuable insights, it has inherent limitations. The resolution of this method may not detect low-abundance taxa that play critical roles in systemic and neurological processes [30]. Additionally, contamination during sample collection and sequencing remains a challenge [31]. To mitigate these issues, we employed stringent protocols, including sterile collection methods, precise DNA extraction techniques, and advanced bioinformatics pipelines. Nonetheless, future work should explore metagenomic or metatranscriptomic approaches for more comprehensive microbiome profiling.

Genetic Factors

This study did not investigate genetic factors that could influence microbiome composition and the risk of cognitive decline. Variants in host genes, particularly those involved in immune response and gut permeability, may play significant roles in shaping microbial populations and mediating their effects on the brain [32]. Future research should incorporate genetic analyses to explore these interactions and provide a more holistic understanding of the microbiome's role in neurodegeneration.

Notes

Ethics Approval

This study protocol was reviewed and approved by the institutional review board of Soonchunhyang University Bucheon Hospital (SCHBC-2020-03-016-002). All participants signed an informed consent form approved by the IRB prior to participation.

Conflicts of Interest

The authors have no conflicts of interest to declare.

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Availability of Data

The datasets are not publicly available but are available from the corresponding author upon reasonable request.

Authors' Contributions

Conceptualization: JYL, ESL; Formal analysis: YP; Funding acquisition: ESL; Investigation: JYL, ESL; Methodology: YP; Project administration: JYL, ESL; Resources: JYL, ESL; Software: YP; Supervision: JYL, ESL; Validation: JYL, ESL; Visualization: YP; Writing–original draft: YP; Writing–review & editing: all authors. All authors read and approved the final manuscript.

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