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Gut microbiota mediates the association between aging and cognitive function: evidence from a population-based Kyotango study linking diet, Bifidobacterium, and Mini-Mental State Examination (MMSE) score

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Abstract

Objective

Age-related cognitive decline is a major public health concern; however, the biological pathways linking aging to cognitive function remain poorly understood. Emerging evidence suggests that gut microbiota may influence brain health; however, human studies integrating clinical factors, diet, microbiota, and cognition remain limited.

Design

We conducted a cross-sectional analysis of community-dwelling older adults (N = 578). Clinical variables, blood biomarkers, and dietary intake were assessed using a validated questionnaire, and gut microbiota profiles (based on 16S rRNA sequencing) were analyzed in relation to cognitive function measured using the Mini-Mental State Examination (MMSE). Multivariable regression models were used to identify independent determinants of MMSE. Mediation analysis was performed to evaluate whether the gut microbiota mediated the association between age and cognitive function. Additional analyses were conducted in a subset excluding participants with suspected dementia (MMSE ≤ 23) to examine diet–microbiota relationships (n = 535).

Results

In multivariable analysis, age, frailty, renal function, inflammatory markers, and specific gut microbial taxa were independently associated with MMSE. Among the microbial features, *Bifidobacterium* showed a significant positive association with cognitive function. Age was inversely associated with *Bifidobacterium*, whereas *Bifidobacterium* was positively associated with MMSE. Mediation analysis demonstrated that *Bifidobacterium* partially mediated the association between age and MMSE (indirect effect: −0.0107, 95% CI: −0.0196 to − 0.0034, p = 0.004). Furthermore, dietary analysis revealed that milk intake was positively associated with *Bifidobacterium*, suggesting a modifiable pathway linking diet, microbiota, and cognition.

Conclusions

Our findings provide evidence that age-related changes in the gut microbiota, particularly reductions in *Bifidobacterium*, partially mediate cognitive decline in older adults. These results support the existence of a diet–microbiota–brain axis and highlight the gut microbiota as a potential target for interventions aimed at preserving cognitive function during aging.

Trial registration number

The UMIN Clinical Trials Registry (UMIN-CTR), UMIN000019486, and UMIN0000 000061232 were registered on December 1, 2015, and April 11, 2026, respectively.

INTRODUCTION

Cognitive decline and dementia are major global health challenges in aging societies. As life expectancy increases, identifying modifiable factors that contribute to the maintenance of cognitive function has become a critical priority [1, 2]. Although established clinical risk factors, including aging, vascular diseases, and metabolic disorders, are known to influence cognitive decline, these factors explain only a limited proportion of the interindividual variability in cognitive function among older adults.

Recent advances in microbiome research have highlighted the gut–brain axis as a key pathway linking peripheral physiology and brain function [3, 4]. The gut microbiota influences host health through multiple mechanisms, including immune regulation, metabolic signaling, and neural communication. Accumulating evidence suggests that alterations in gut microbial composition are associated with aging-related conditions, including cognitive decline [5, 6]. However, most existing evidence is derived from experimental models or small clinical studies, and integrated analyses that incorporate clinical variables, diet, microbiota, and cognitive outcomes in population-based cohorts remain limited.

Among the gut microbial taxa, *Bifidobacterium* has attracted particular attention as a potential mediator of healthy aging [7]. *Bifidobacterium* species exert anti-inflammatory effects, produce short-chain fatty acids and other neuroactive metabolites, and contribute to gut barrier integrity. Notably, *Bifidobacterium* abundance decreases with age [8], suggesting a potential link between age-related microbiota alterations and cognitive decline. Despite these observations, it remains unclear whether *Bifidobacterium* independently contributes to human cognitive function and plays a mediating role in the relationship between aging and cognition.

Diet is a major determinant of the composition and function of the gut microbiota [9, 10]. Dietary components can shape microbial communities and their metabolic outputs, thereby influencing host physiology and brain function. In particular, dietary factors such as dairy products, fiber, and fermented foods have been associated with an increased abundance of beneficial microbes, including *Bifidobacterium*. However, the specific dietary factors that modulate *Bifidobacterium* and their relevance to cognitive function in older populations have not yet been fully elucidated. Therefore, understanding these relationships in real-world populations is essential. Kyotango, a coastal region in Japan, is characterized by exceptional longevity and traditional dietary patterns, providing a unique setting to investigate the interactions among diet, microbiota, and cognitive function in the context of healthy aging.

In this study, we performed an integrated analysis of clinical variables, blood biomarkers, dietary intake, and gut microbiota in a community-based cohort of older adults from Kyotango. First, we identified clinical determinants of cognitive function. We then examined the association between the gut microbiota and cognitive function, with a particular focus on *Bifidobacterium*. To minimize potential bias

in the dietary assessment, analyses involving diet were conducted after excluding participants with suspected dementia. Finally, we investigated the dietary factors associated with *Bifidobacterium* and evaluated whether *Bifidobacterium* mediates the relationship between aging and cognitive function.

METHODS

Study design and participants

This study was a cross-sectional analysis conducted as part of the Kyotango Multi-purpose Cohort Study, initiated by the Department of Longevity and Community Epidemiology at Kyoto Prefectural University of Medicine. It involved 587 community-dwelling residents aged ≥ 65 years from the Kyotango area in northern Kyoto Prefecture who had available gut microbiota information and dietary and nutrient intake data. The Kyotango region, comprising two cities and two towns, has a population of less than 100,000, with over 38% of the residents aged 65 years or older, making it one of the most aged regions in Kyoto Prefecture [11]. Despite its aging population, the region is known for its high number of centenarians (more than 200 per 100,000 individuals), making it a notable area for longevity in Japan.

Analysis of cognitive function

Cognitive function was assessed using the Mini-Mental State Examination (MMSE), a widely used screening tool for cognitive impairment, and the total score was treated as a continuous variable in all analyses [12, 13]. Participants were categorized into three groups based on established cutoff values: normal cognitive function (MMSE ≥ 28), mild cognitive impairment (MCI; MMSE 24–27), and suspected dementia (MMSE ≤ 23). These thresholds are commonly used in epidemiological studies to stratify older adults' cognitive status. The MMSE has been validated in the Japanese population and demonstrates adequate reliability and diagnostic performance for cognitive impairment [14].

Analysis of the fecal microbiota

Fecal microbial DNA was extracted with a Maxwell® RSC Fecal Microbiome DNA Kit (Promega, Tokyo, Japan) according to the manufacturer's instructions. The V3–V4 region of the 16S rRNA gene was amplified using the primer set consisting of 341F (5'-CCTACGGGNGGCWGCAG-3') and 805R (5'-GACTACHVGGGTATCTAATCC-3') with Tks Gflex DNA Polymerase (TaKaRa Bio, Kusatsu, Japan). PCR amplification was performed using the following thermal cycling conditions: an initial denaturation at 95 °C for 3 min, followed by 25 cycles consisting of denaturation at 95 °C for 30 s, annealing at 55 °C for 30 s, and extension at 72 °C for 30 s, concluding with a final elongation step at 72 °C for 5 min. The resulting amplicons were purified using NucleoFast96 PCR plates (TaKaRa Bio), and a subsequent indexing PCR was carried out using unique dual-index primer sets compatible with MiSeq sequencing, following Illumina's standard protocol (Illumina, San Diego, CA, USA). After indexing, PCR products were purified, normalized using the SequalPrep Normalization Plate Kit (Life Technologies, Tokyo, Japan), and pooled

at equimolar concentrations. The pooled library was purified using AMPure XP magnetic beads (Beckman Coulter, Brea, CA, USA). The resulting purified library was subjected to 285-bp paired-end sequencing on the Illumina MiSeq platform using the MiSeq Reagent Kit v3. Data obtained from MiSeq sequencing were analyzed as described by Miura et al. [15], with some exceptions. In this study, the version of QIIME2 [16] used was version 2024.5, and the taxonomy of amplicon sequence variants (ASVs) was assigned against SLIVA 138 [17]. The raw 16S rRNA gene sequencing data generated in this study have been deposited in the NCBI Sequence Read Archive (SRA) under BioProject accession numbers PRJNA1470357 and PRJNA1440982. During peer review, the datasets can be accessed using the following reviewer links: PRJNA1440982 (770 samples):

https://dataview.ncbi.nlm.nih.gov/object/PRJNA1440982?reviewer=34krs7kauassc617o1nk4edt22 and PRJNA1470357 (20 samples):

https://dataview.ncbi.nlm.nih.gov/object/PRJNA1470357?reviewer=km535m01812ji7midd1q2j6mcs . All other relevant the Editor. All other relevant data supporting the findings of this study are included within the article and its Supplementary information files.

The relative abundance data at the genus level were transformed using a centered log-ratio (CLR) transformation to account for compositionality. All microbiota analyses were performed using CLR-transformed and z-standardized genus-level data.

Assessment of dietary and nutrient intakes

A brief self-administered diet history questionnaire (BDHQ) was used to assess dietary and nutrient intakes [18]. Because dietary assessment based on self-reported questionnaires may be influenced by cognitive function, participants with suspected dementia (MMSE ≤ 23) were excluded from the dietary analysis to minimize potential reverse causation. Subsequent analyses were conducted among participants with normal cognition or mild cognitive impairment (MCI). In addition, individuals with an implausible total energy intake (< 600 kcal/day or > 4,000 kcal/day) were excluded from the analysis. The dietary intake of individual food items and nutrients was energy-adjusted using the density method and expressed per 1,000 kcal of the total energy intake. The energy-adjusted values were further standardized as z-scores based on the population mean and standard deviation, and these standardized values were used for the statistical analyses.

Ethics statements

The study conformed to the ethical principles of the Declaration of Helsinki, and the research protocol was approved by the Ethics Committee of Kyoto Prefectural University of Medicine (approval numbers: ERB-C-885-9 and ERB-C-2182-1). All participants provided written informed consent prior to enrollment. This study was registered with the UMIN Clinical Trials Registry (UMIN-CTR), UMIN000019486 and UMIN0000 000061232 on December 1, 2015, and April 11, 2026, respectively.

Statistics

Correlation analysis. Spearman's rank correlation coefficients (ρ) were calculated to assess associations between MMSE scores and clinical variables, blood biomarkers, and microbiota features. Given the large number of comparisons, multiple-testing corrections were performed using the Benjamini–Hochberg false discovery rate (FDR) method. The corresponding q-values were calculated, and an FDR-adjusted threshold of $q < 0.05$ was considered statistically significant.

Multivariable linear regression analysis. Multivariable linear regression analysis was performed to identify independent determinants of cognitive function (MMSE score as a continuous outcome). Candidate variables identified from the univariate analyses ($p < 0.20$) were entered into the model using a stepwise selection procedure, with entry and removal thresholds set at $p < 0.20$. Regression results are presented as unstandardized coefficients (B), standardized coefficients (β), standard errors (SE), 95% confidence intervals (CIs), and p-values. Model performance was evaluated using the coefficient of determination (R^2). Multicollinearity was assessed using variance inflation factors (VIFs), with a threshold of VIF > 10 considered indicative of significant multicollinearity. In cases of collinearity (e.g., between cystatin C and Cys-eGFR), biologically interpretable variables were prioritized for inclusion in the final model. Microbiota-related variables were included in the same regression framework as the clinical and biochemical variables to evaluate their independent contributions.

Group comparisons. The participants were categorized into three groups based on MMSE scores: normal cognitive function (MMSE ≥ 28), mild cognitive impairment (MCI; MMSE 24–27), and suspected dementia (MMSE ≤ 23). Group differences in continuous variables were assessed using analysis of variance (ANOVA) or the Kruskal–Wallis test, depending on the data distribution. Post hoc comparisons were performed using the Tukey-Kramer or Dunn's test, as appropriate. Categorical variables were compared using the chi-square test.

Mediation analysis. Mediation analysis was performed to assess whether the gut microbiota mediated the association between aging (or dietary factors) and cognitive function. The mediation framework included the following paths: (a) association between exposure and mediator; (b) association between mediator and outcome adjusted for exposure; (c) total effect of exposure on outcome; and (d) direct effect after adjusting for the mediator. The indirect effects ($a \times b$) were estimated using a nonparametric bootstrap approach with 5,000 resamples. Bias-corrected 95% confidence intervals (CIs) were also calculated. The mediation effect was considered statistically significant if the confidence interval did not include zero. All mediation models were adjusted for the relevant covariates identified in the multivariable regression analyses, including age, sex, frailty, education level, and inflammatory markers, as appropriate.

Software and reproducibility. All statistical analyses were performed using ChatGPT-assisted Python (version 3.10; Python Software Foundation, Wilmington, DE, USA). Data processing and visualization were performed using standard scientific computing libraries, including pandas, numpy, scipy,

statsmodels, matplotlib, and seaborn. After using this tool/service, the authors reviewed and edited the content as required and took full responsibility for the content of the published work. All analyses were conducted in accordance with established statistical and microbiome analysis guidelines to ensure reproducibility and robustness of the results.

RESULTS

Association between Cognitive Function and Clinical Factors

Among the 578 participants, 43 (7.4%) were classified as having suspected dementia (MMSE ≤ 23), 255 (44.1%) as mild cognitive impairment (MCI; MMSE 24–27), and 280 (48.4%) as normal cognitive function (MMSE ≥ 28), indicating that approximately half of the cohort exhibited some degree of cognitive impairment. Background clinical variables and blood biomarkers are shown in Table 1. Spearman correlation analyses identified several clinical and blood variables associated with MMSE (Supplementary Tables S1 and S2). Among these, age showed the strongest negative correlation (Spearman's $\rho = -0.242$, $p < 0.001$) (Supplementary Fig. 1). Other variables significantly associated with lower MMSE scores included frailty, sarcopenia, and IL-6 levels, whereas cystatin C–based estimated glomerular filtration rate (Cys-eGFR) and lymphocyte percentage were positively associated with MMSE. However, the magnitude of these correlations was modest ($|\rho| \leq 0.24$).

After FDR correction, only a limited number of variables remained statistically significant, including age, frailty, sarcopenia, and IL-6 levels, suggesting that robust clinical correlates of MMSE were relatively few. In multivariable linear regression analysis, age remained an independent determinant of MMSE ($\beta = -0.0875$, $p < 0.001$). In addition, frailty ($\beta = -0.855$, $p = 0.006$), IL-6 ($\beta = -0.058$, $p = 0.004$), and education level ($\beta = -0.477$, $p = 0.042$) were independently associated with MMSE. By contrast, sarcopenia and Cys-eGFR were not independently associated after adjustment. The overall explanatory power of the model was modest ($R^2 = 0.116$), indicating that a substantial proportion of the variability in cognitive function could not be explained by conventional clinical factors.

Association between Cognitive Function and Gut Microbiota

Next, we investigated the association between cognitive function and the composition of the gut microbiota. Spearman correlation analyses were performed between MMSE scores and gut microbiota-related variables, including alpha diversity indices and genus-level abundance data (CLR-transformed and standardized z-scores), followed by false discovery rate (FDR) correction. The results are summarized in Supplementary Tables S3 and S4. Several taxa showed nominal associations with MMSE; however, after FDR correction, only *g__Bifidobacterium* remained significantly associated with MMSE (Spearman's $\rho = 0.141$, $p < 0.001$, FDR $q = 0.017$) (Fig. 2a). Several other taxa, including f__*Enterobacteriaceae*, f__*Lachnospiraceae, g__Blautia, and* g__Subdoligranulum, showed borderline

associations but did not reach statistical significance after multiple-testing corrections. To further illustrate this association, participants were stratified into three groups based on MMSE scores: normal (MMSE ≥ 28), mild cognitive impairment (MCI; MMSE 24–27), and suspected dementia (MMSE ≤ 23). As shown in Fig. 2b, the relative abundance of *Bifidobacterium* was significantly lower in both the MCI and suspected dementia groups than in the control group ($p < 0.05$ and $p < 0.01$, respectively; Tukey–Kramer HSD test). These findings indicate that, among the gut microbiota-related variables examined, *Bifidobacterium* showed the most robust and consistent association with cognitive function.

Independent Determinants of Cognitive Function

To identify independent determinants of cognitive function, we performed multivariable linear regression analysis, including clinical variables, blood biomarkers, and gut microbiota variables that met the screening criteria ($p < 0.20$ in univariate analyses). The results are summarized in Supplementary Table S5 and shown in Fig. 3.

In the final model, several factors were independently associated with MMSE. Age was significantly and negatively associated with cognitive function (standardized $\beta = -0.156$), indicating that advancing age was a major determinant of lower MMSE scores. Similarly, frailty showed a significant negative association (standardized $\beta = -0.159$), supporting the link between physical vulnerability and cognitive decline. Among blood biomarkers, IL-6 was independently associated with lower MMSE (standardized $\beta = -0.106$), suggesting a potential role of systemic inflammation in cognitive impairment. In contrast, renal function, represented by Cys-eGFR, was positively associated with MMSE (standardized $\beta = 0.223$), indicating that better kidney function was linked to preserved cognitive performance. Cystatin C was excluded from the final model because of its collinearity with the Cys-eGFR.

Importantly, among the gut microbiota variables, *g__Bifidobacterium* remained independently associated with higher MMSE scores (standardized $\beta = 0.139$), even after adjusting for age, frailty, inflammation, and renal function. Other genera, including *g__Lachnospira* (standardized $\beta = 0.090$) and *g__Prevotella* (standardized $\beta = 0.079$), showed weaker but positive associations with MMSE. In addition, several hematological and biochemical parameters, including red blood cell count (RBC; standardized $\beta = 0.119$), immunoglobulins (IgM: standardized $\beta = 0.101$; IgA: standardized $\beta = -0.094$), and lactate dehydrogenase (LD; standardized $\beta = 0.093$), were retained in the model, although their contributions were relatively modest.

The forest plot (Fig. 3) illustrates the magnitude and direction of these associations, highlighting that traditional clinical factors (age and frailty), systemic inflammation (IL-6), and the gut microbiota (particularly *Bifidobacterium*) independently contribute to cognitive function. Notably, the independent association with *Bifidobacterium* suggests that the gut microbiota may play a role in cognitive aging beyond conventional risk factors. These findings suggest that gut microbiota, particularly *Bifidobacterium*, is an independent and potentially modifiable factor associated with cognitive function in older adults.

Associations between Gut Microbiota and Dietary and Nutritional Factors

Because dietary intake assessed by the BDHQ may be influenced by cognitive function, we conducted subsequent analyses after excluding participants with suspected dementia (MMSE ≤ 23) to minimize potential reverse causation. Spearman correlation analyses with false discovery rate (FDR) correction were performed to evaluate the associations between dietary variables and *Bifidobacterium* abundance. Among food items, milk intake showed a significant positive correlation with *Bifidobacterium* (Spearman's ρ = 0.146, FDR q = 0.010) (Fig. 4a). Among the nutritional components, several fatty acids showed strong and consistent positive correlations with *Bifidobacterium*. In particular, medium-chain and related fatty acids, including C10:0 (ρ = 0.204, q = 0.00017), C8:0, C10:1, and C12:0, were significantly associated with *Bifidobacterium*, with correlation coefficients consistently ranging from approximately 0.19 to 0.20 (Fig. 4b).

Mediation Analysis

Mediation analysis was performed to evaluate whether *Bifidobacterium* mediates the association between age and cognitive function (MMSE) (Fig. 5a**)**. Age was significantly and negatively associated with *Bifidobacterium* (a path: β = −0.035, p < 0.001). In turn, *Bifidobacterium* was positively associated with MMSE after adjustment for age (b path: β = 0.307, p = 0.004). The direct effect of age on MMSE remained significant after inclusion of *Bifidobacterium* in the model (c' path: β = −0.110, p < 0.001). Importantly, the indirect effect of age on MMSE through *Bifidobacterium* was statistically significant (indirect effect = − 0.0107, 95% CI: −0.0196 to − 0.0034), indicating partial mediation. These findings suggest that age-related decline in *Bifidobacterium* contributes, at least in part, to the deterioration of cognitive function.

We next examined whether *Bifidobacterium* mediates the association between milk intake and cognitive function in participants without suspected dementia (Fig. 5b). Milk intake was positively associated with *Bifidobacterium* (a path: β = 0.164, p = 0.0002). However, the association between *Bifidobacterium* and MMSE, adjusted for milk intake, showed only a non-significant trend (b path: β = 0.160, p = 0.072). The direct effect of milk intake on MMSE remained significant (c' path: β = 0.211, p = 0.019). The indirect effect of milk intake on MMSE through *Bifidobacterium* did not reach statistical significance (indirect effect = 0.026, 95% CI: −0.002 to 0.065), as the confidence interval included zero. These findings indicate that while milk intake is associated with both *Bifidobacterium* abundance and cognitive function, the mediating role of *Bifidobacterium* in this pathway is not statistically supported.

DISCUSSION

In this population-based study of older adults from a long-lived region in Japan, we demonstrated that conventional clinical factors explained only a limited proportion of the variability in cognitive function, whereas gut microbiota, particularly *Bifidobacterium*, emerged as a robust and independent determinant.

Importantly, our findings provide evidence supporting a microbiota-mediated pathway that links aging to cognitive function. Although aging was the strongest determinant of MMSE, its explanatory power was modest, even when combined with frailty, inflammation, and education level. This observation highlights a critical gap in our understanding of cognitive aging and underscores the need to identify additional biological mechanisms beyond traditional risk factors.

The association between *Bifidobacterium* spp. and cognitive function is biologically plausible. Bifidobacteria are known to exert anti-inflammatory effects, modulate immune responses, and produce metabolites such as short-chain fatty acids and neuroactive compounds that influence the gut–brain axis [4, 19]. A randomized double-blind placebo-controlled trial demonstrated that B. breve supplementation improved cognitive function in older adults with mild cognitive impairment [20]. These properties indicate that *Bifidobacterium* is a key microbial taxon involved in maintaining brain homeostasis during aging processes.

A central finding of this study is that *Bifidobacterium* partially mediates the association between aging and cognitive function. This mediation effect suggests that age-related alterations in the gut microbiota composition may represent a mechanistic link between aging and cognitive decline. Given that the abundance of *Bifidobacterium* decreases with age [8], our results support a model in which the loss of beneficial microbial taxa contributes to cognitive function deterioration. This concept aligns with the framework of “microbiome aging,” in which age-associated shifts in microbial ecosystems influence systemic physiology [21].

Diet appears to be a key upstream determinant of this pathway. In our cohort, milk intake showed a consistent positive association with *Bifidobacterium*, suggesting that dietary habits may modulate microbiota composition and influence cognitive function. Although mediation analysis did not demonstrate a statistically significant indirect effect of *Bifidobacterium* on the milk–MMSE pathway, the observed associations support the existence of a diet–microbiota–brain axis [10]. These findings are consistent with previous epidemiological and interventional studies, indicating that dairy intake and *Bifidobacterium* supplementation may contribute to cognitive health [22, 23].

Another factor that may explain the observed association between milk intake and *Bifidobacterium* abundance is host genetic background [24]. In East Asian populations, including Japanese individuals, lactase non-persistence is highly prevalent because of genetic variants in LCT [25]. As a result, a substantial proportion of the ingested lactose is not fully digested in the small intestine and reaches the colon, where it can serve as a substrate for lactose-utilizing bacteria such as *Bifidobacterium*. Previous studies have demonstrated that lactase gene variants are associated with an increased abundance of *Bifidobacterium* in the Japanese population [24]. These findings highlight the importance of integrating host genetics, diet, and microbiota to better understand inter-individual variability in cognitive aging. This gene–diet–microbiota interaction provides a biologically plausible explanation for the positive association between milk intake and Bifidobacterium observed in our study, and suggests that the impact of dietary factors on the gut microbiota may be modulated by the host genetic background. Such

population-specific mechanisms should be considered when interpreting diet–microbiota relationships in human studies.

The Kyotango cohort provided a unique context for interpreting these results. As a region characterized by exceptional longevity and traditional dietary patterns, this population may represent a model of successful aging in which host–microbiome–diet interactions are optimized. Our findings suggest that preservation of a “youth-associated” microbiota profile, characterized by higher *Bifidobacterium* abundance, may be a key feature of healthy cognitive aging.

This study had several limitations. The cross-sectional design precludes causal inferences, and residual confounding factors cannot be excluded. Dietary assessment was based on self-reported data, although potential bias was minimized by excluding participants with suspected dementia. Additionally, functional and metabolomic profiling of the microbiome was not performed, which may provide further mechanistic insights for future research.

In conclusion, we identified *Bifidobacterium* as a key microbial taxon that links aging and cognitive function in humans. These findings support a model in which age-related alterations in the gut microbiota contribute to cognitive decline and highlight the gut microbiota as a potentially modifiable target for interventions aimed at preserving cognitive function in aging populations.

Declarations

Ethics approval and consent to participate

The study conformed to the ethical principles of the Declaration of Helsinki, and the research protocol was approved by the Ethics Committee of Kyoto Prefectural University of Medicine (approval numbers: ERB-C-885-9 and ERB-C-2182-1). All participants provided written informed consent prior to enrollment. This study was registered with the UMIN Clinical Trials Registry (UMIN-CTR), UMIN000019486 and UMIN0000 000061232 on December 1, 2015, and April 11, 2026, respectively.

Consent for publication

Not applicable

Availability of data and materials

The raw 16S rRNA gene sequencing data generated in this study have been deposited in the NCBI Sequence Read Archive (SRA) under accession numbers PRJNA1470357 and PRJNA1440982. All other relevant data supporting the findings of this study are included within the article and its supplementary information files.

Competing interests

YN received scholarship funds from Taiyo Kagaku Co. Ltd., Morinaga Co. Ltd., Miyarisan Pharma Co. Ltd, Morishita-Jintan Co. Ltd., Fujikko Co. Ltd., Mizkan Co. Ltd., Mykinso Co. Ltd. and KINS Co. Ltd.; a collaboration research fund from TOTO Ltd., Takeda Pharma. Co. Ltd. and Taiyo Kagaku Co., Ltd.; and received lecture fees by Takeda Pharma. Co. Ltd., Biofermin. Co. Ltd., and Miyarisan Pharma. Co. Ltd. TT received a collaboration research funds from PreMedica Inc., and lecture fees from Mochida Pharma. Co. Ltd., Yanssen Pharmaceutical K.K., EA Pharma. Co. Ltd., Mitsubishi Tanabe Pharma Corporation, Takeda Pharmaceutical Co. Ltd, AbbVie GK, Bristol-Myers Squibb, and Pfizer Japan Inc.

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Authors' contributions

Conceptulization, Y.N. and T.T.; validation and formal analysis, T.Y., H.K., K.M.; and R.I.; human cohort study management, N.O., A.A., T.K., J.N., T.K. and S.M.; writing-original draft preparation, Y.N.; writing-review and editing, K.M., R.I., and T.T.; funding acquisition, Y.N. and S.M.. All authors have read and agreed to be published version of the manuscript.

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Table

Tables 1 is available in the supplementary files section.

Figures

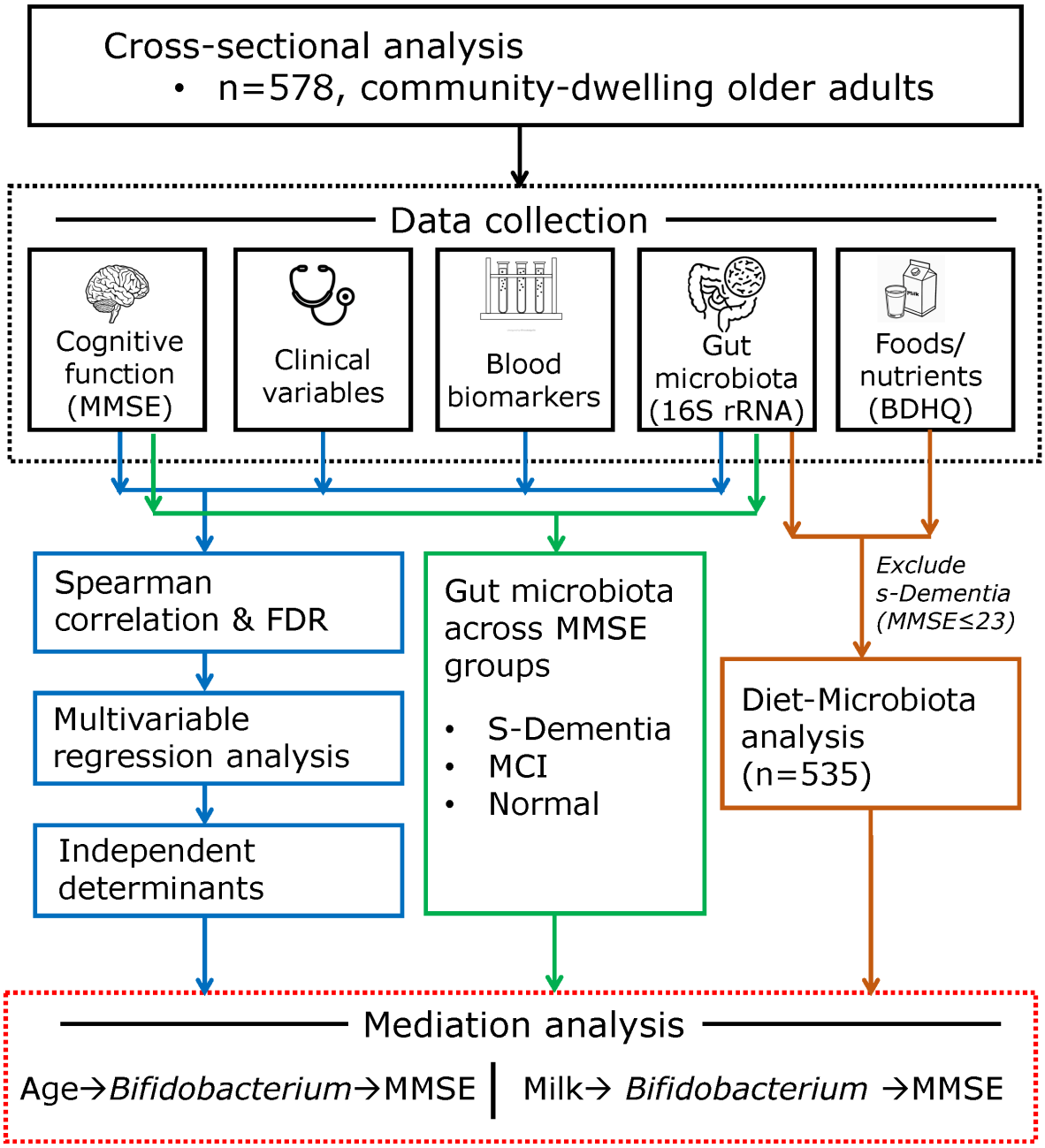

Figure 1

Figure 1

Study design and analytical workflow.

Flow diagram illustrating the study population and analytical strategy. A total of 578 community-dwelling older adults were included in the primary analyses of clinical variables, blood biomarkers, gut microbiota, and cognitive function as assessed using the MMSE. For diet–microbiota analyses, participants with

suspected dementia (MMSE ≤ 23) were excluded (n = 535) to minimize potential bias in self-reported dietary assessment. Statistical analyses included Spearman's correlation with false discovery rate (FDR) correction, multivariable linear regression, and mediation analysis.

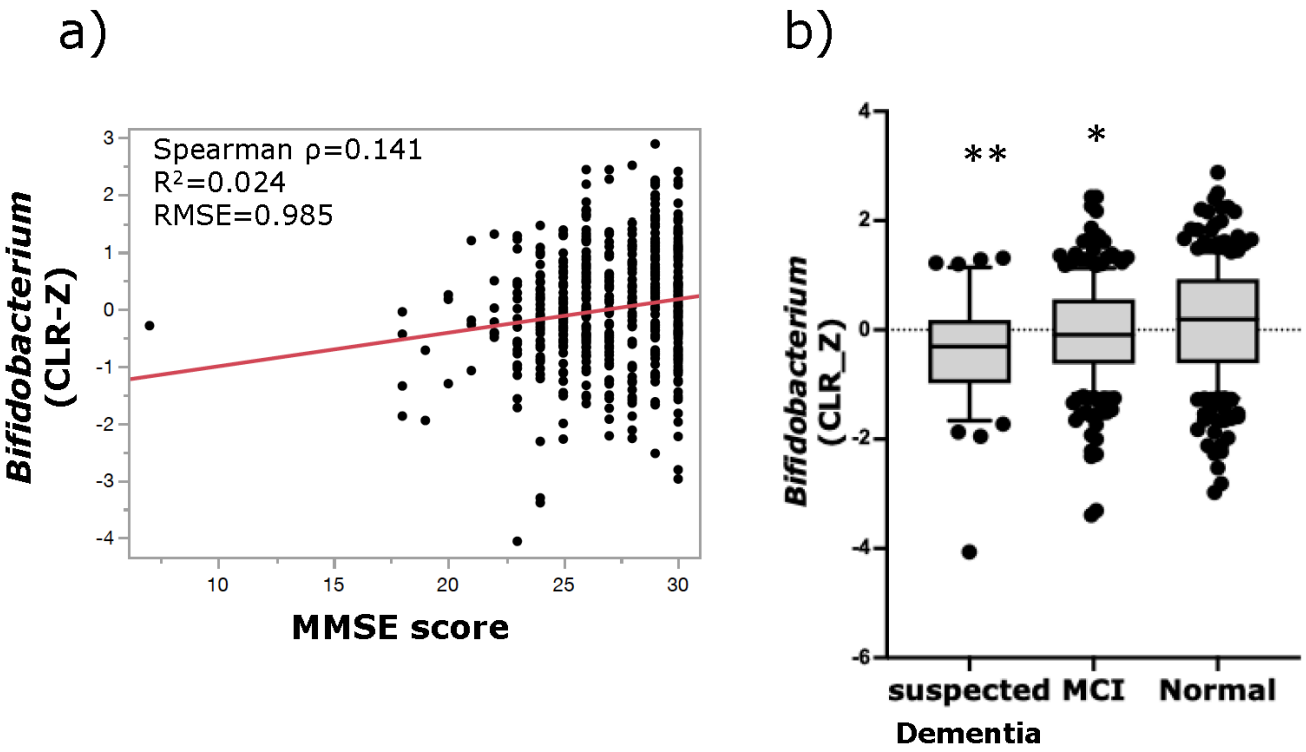

Figure 2

Association between *Bifidobacterium* abundance and cognitive status.

Comparison of the relative abundance of *Bifidobacterium* across three cognitive groups: normal (MMSE ≥ 28), mild cognitive impairment (MCI; MMSE 24–27), and suspected dementia (MMSE ≤ 23). The data are presented as box plots. *Bifidobacterium* abundance was significantly lower in the MCI and suspected dementia groups than in the control group (Tukey–Kramer HSD test). These results demonstrate a graded association between reduced *Bifidobacterium* levels and cognitive decline.

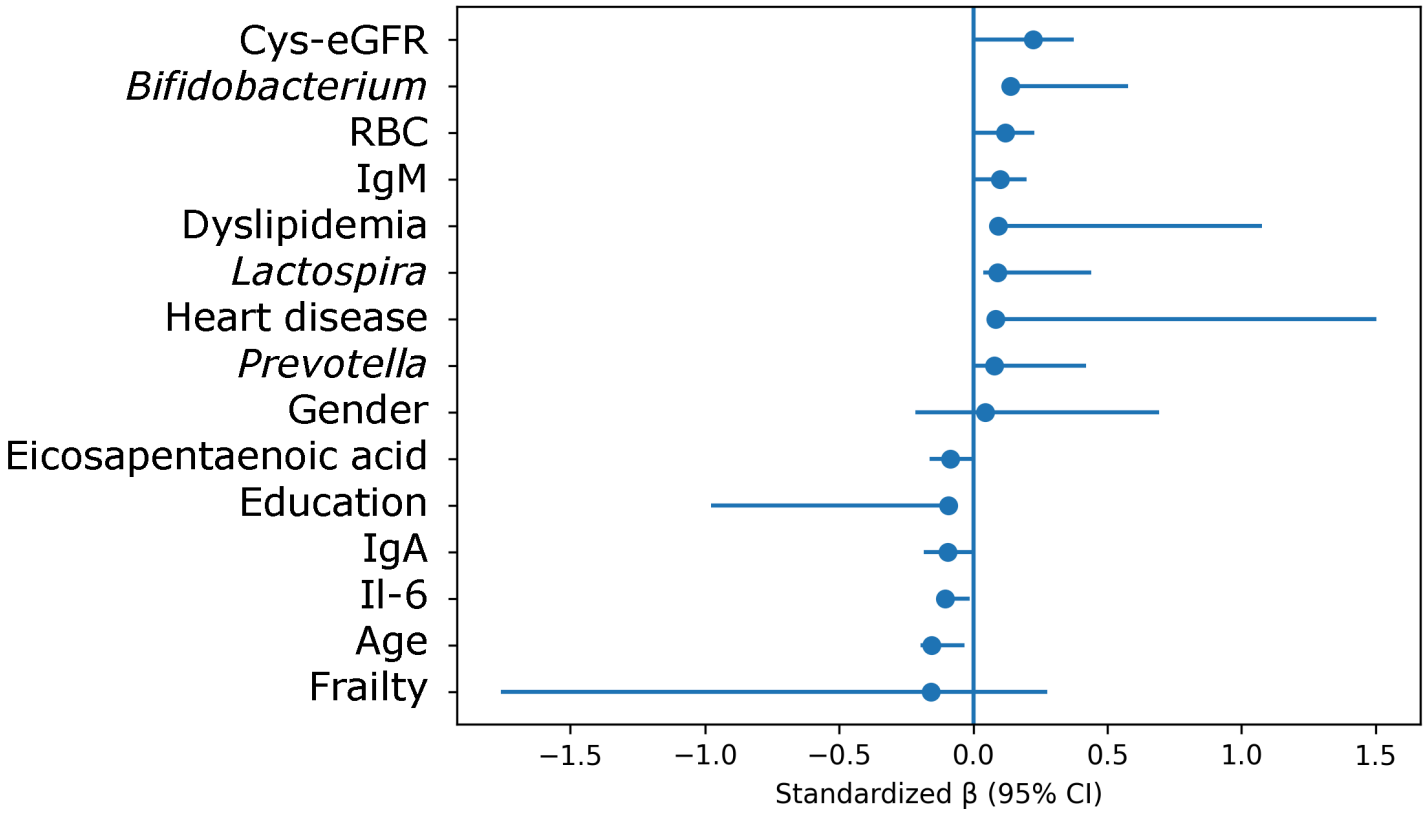

Figure 3

Forest plot of independent determinants of cognitive function.

Forest plot showing standardized regression coefficients (β) and standard errors (SE) from the final multivariable model for MMSE. Variables were selected using stepwise regression (entry/removal, p = 0.20). Clinical factors (age, frailty, education level, and IL-6) and gut microbiota (*Bifidobacterium*) were independently associated with MMSE. The model demonstrated modest explanatory power (R^2 = 0.116), indicating that cognitive function was influenced by multiple factors.

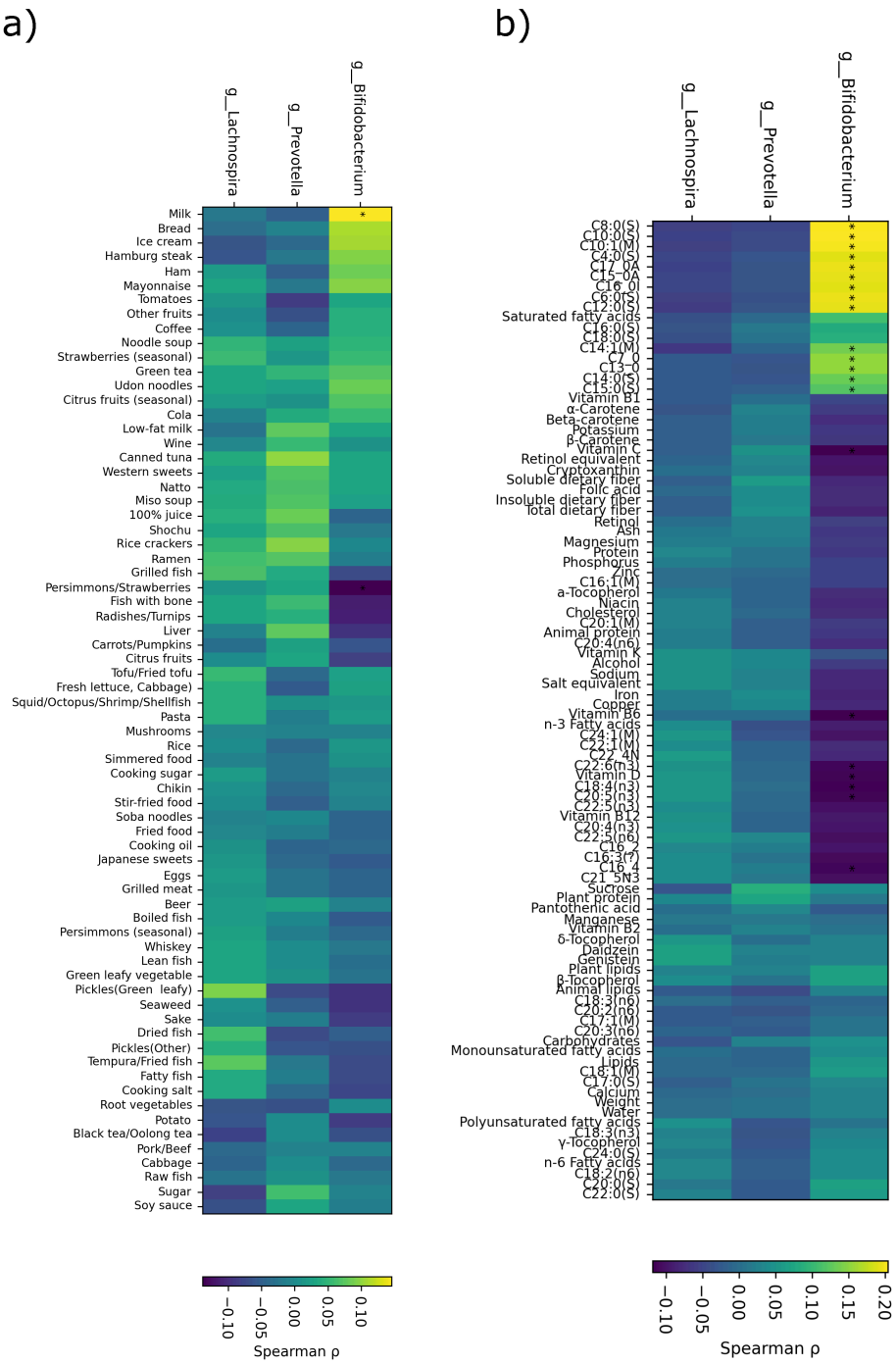

Figure 4

Figure 4

Associations between dietary factors and gut microbiota.

Heatmap showing Spearman's correlation coefficients between dietary variables (energy-adjusted and z-standardized) and key gut microbial genera (*Bifidobacterium*, *Prevotella*, and *Lachnospira*) in participants without suspected dementia (n = 535). Hierarchical clustering was applied to both axes. Color intensity

represents the strength and direction of the correlation. Milk intake and several fatty acids showed positive associations with *Bifidobacterium* after FDR correction.

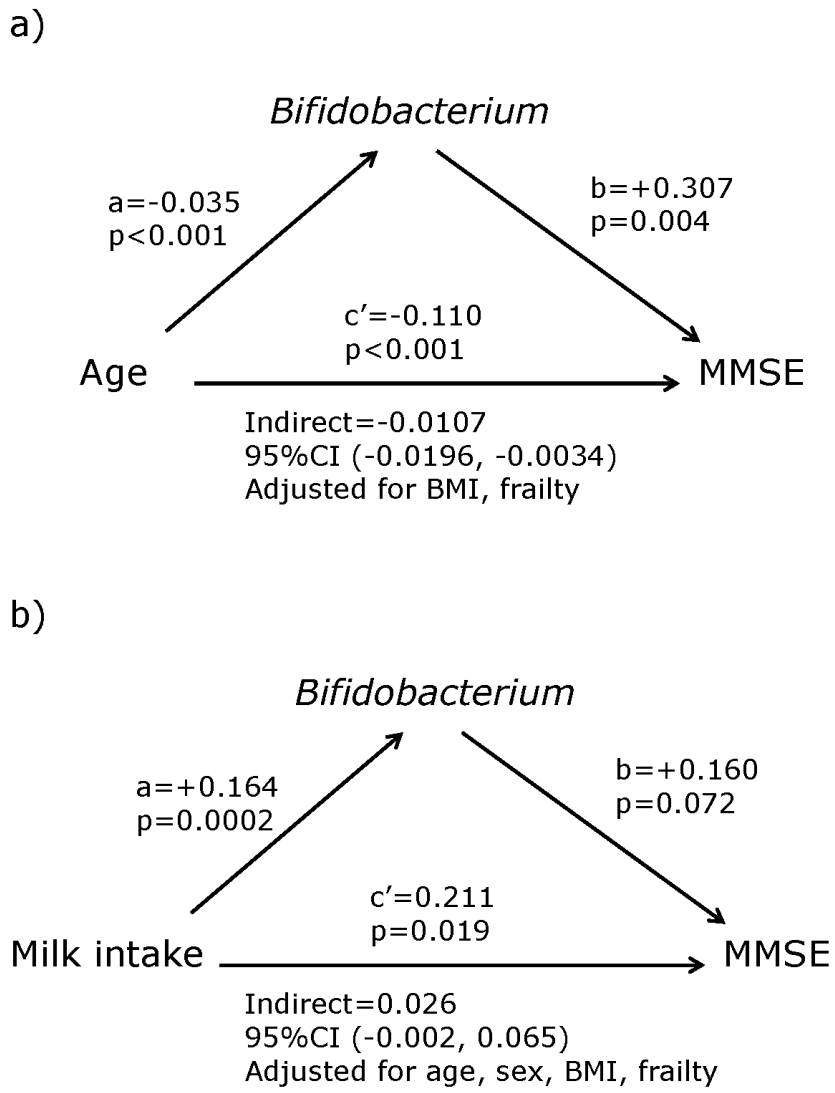

Figure 5

Figure 5

Mediation analyses linking age and milk intake to cognitive function through *Bifidobacterium*.
(A) Mediation model evaluating whether *Bifidobacterium* mediates the association between age and

cognitive function (MMSE). Age was negatively associated with *Bifidobacterium* (a path), whereas *Bifidobacterium* was positively associated with MMSE (b path). The direct effect of age on MMSE (c' path) remained significant after adjustment for covariates. The indirect effect (a × b) was statistically significant based on bootstrap analysis, indicating that *Bifidobacterium* partially mediates the association between age and cognitive function.

(B) Mediation model evaluating whether *Bifidobacterium* mediates the association between milk intake and MMSE in participants without suspected dementia (MMSE ≤ 23 excluded). Milk intake was positively associated with *Bifidobacterium* (a path) and MMSE (total effect, c path). Although the direct effect of milk on MMSE (c' path) remained significant after adjustment, the indirect effect through *Bifidobacterium* did not reach statistical significance, as the 95% confidence interval included zero.

All models were adjusted for the relevant covariates identified in the multivariable analyses, including age, sex, frailty, education level, and IL-6 level, as appropriate. Indirect effects were estimated using bootstrap resampling with 5,000 iterations.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Table1.pdf
- SupplementaryFigureS1.pdf
- SupplenetaryTableS1.pdf
- SupllementaryTableS2.pdf
- SupplementaryTableS3.pdf
- SupplementaryTableS4.pdf
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