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Plasma acetic acid mediates the relationship between gut microbiome and various health measures in older adults

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Keywords: short-chain fatty acid; acetic acid; gut microbiota; lipid profile; body composition; gray matter volume; cognitive performance

Abstract

Background: Short-chain fatty acids are believed to mediate microbiome-host interactions. Acetic acid is the most abundant systemic short-chain fatty acid, but knowledge about its physiological functions comes mainly from rodent experiments, with limited human research particularly in the aging population.

Methods: In this cross-sectional observational study, we examined the association between the gut microbiota and plasma acetic acid, specifically investigating the mediating effect of plasma acetic acid on the relationship between the gut microbiota and blood lipid profile, body composition, brain gray matter volume, and cognitive performance in older adults. The gut microbiome was profiled using full-length 16S rRNA gene sequencing to enable taxonomic classification.

Results: Here we show that specific gut microbial co-abundance group is associated with plasma acetic acid levels. Higher plasma levels of acetic acid are associated with lower plasma triglyceride levels, higher high-density lipoprotein cholesterol levels, lower body mass index, lower body fat mass, higher thalamic volume, and higher cognitive performance in certain domains. Additionally, we show that plasma acetic acid mediates the relationship between gut microbiome on these health measures.

Conclusions: This study identifies gut microbial group linked to plasma acetic acid and demonstrates its potential mediating role between the gut microbiome, blood lipid profile, brain volume and cognitive function in older adults. These insights pave the way for future research and highlight the potential of acetic acid as an intervention target for metabolic and neurological diseases, contributing to strategies that promote healthy aging.

Plain language summary

The gut microbiome plays an important role in maintaining human health, partly through producing short-chain fatty acids such as acetic acid. However, little is known about how acetic acid relates to metabolic and brain health in older adults. In this study, we examined stool and blood samples from older individuals to assess the relationship between gut bacteria, plasma acetic acid levels, and various health measures, including body composition, blood lipids, brain structure, and cognitive performance. We found that higher plasma acetic acid levels were associated with healthier metabolism, greater brain volume, and better cognitive abilities. These results suggest that acetic acid may serve as a link between gut health and metabolic health as well as brain function during aging, highlighting its potential as a target for future preventive strategies.

Introduction

The world is undergoing a demographic transition characterized by rapid population aging, driven by increasing life expectancies and declining birth rates. As advances in healthcare and improvement in living standards continue to extend human longevity, the proportion of older adults in many countries is increasing at an unprecedented rate. In Taiwan, for instance, the percentage of elderly population is projected to exceed 20% by 2025, classifying it as a super-aged society. However, the growing aging population has led to a higher prevalence and burden of age-associated morbidities, such as obesity, cardiovascular disease, diabetes, and dementia, which present new challenges that healthcare systems struggle to keep pace with.

Ongoing research is actively exploring solutions to mitigate or forestall age-associated illnesses from various perspectives, one of which involves exploring the insights from the gut microbiota. Compelling evidence from epidemiological and omics-based researches, complemented by cellular studies and animal experiments, indicates that human gut microbiome has profound impact on a multitude of physiological processes, ranging from metabolism, immune regulation, and neurologic functions as well as endocrine pathways [1-5]. Studies conducted in recent years have identified several associations between gut microbiome composition and various measures of health in the aging population [6], including physical fitness [7], fragility [8,9], and survival [10]. Understanding microbiome-host interactions can uncover potential therapeutic targets and contribute to personalized interventions to improve health and quality of life in older adults.

In older adults, the abundance, diversity, and composition of the gut microbiota differ substantially from those in younger adults [11,12]. One of the consistent signatures of gut microbiota alterations in the aging population is the reduction of short-chain fatty acid (SCFA)-producing bacteria, such as

Coprococcus and *Faecalibacterium prausnitzii* [13-22]. SCFAs are an important class of bioproducts that play an essential role in microbe-host metabolic crosstalk [23,24]. They are saturated aliphatic organic acids with 1 to 6 carbon atoms derived from the gut microbiota through saccharolytic fermentation of dietary fiber and resistant starches [25,26]. Acetic acid, propionic acid, and butyric acid are the primary SCFAs produced by the gut microbiota, with acetic acid being the most abundant systemic SCFA [27]. Animal studies have shown that acetic acid can act as a regulatory agent, influencing lipid and energy metabolism [28-31]. In the central nervous system, acetic acid has been found to support brain metabolic well-being by modulating microglial function, both in healthy states and in the context of neurodegeneration [32]. Depletion of acetate-producing bacteria in the gut microbiota under experimental conditions affected the gut-brain neural pathway and accelerated cognitive dysfunction in diabetic mice [33]. Investigating this microbiome-metabolic crosstalk pathway in older adults is crucial for understanding the potential impact of age-related alterations in the gut microbiome and the resulting functional modifications on metabolism and health.

Although *in vitro* and animal studies have provided valuable insights into the biological functions of acetic acid, evidence from human research—particularly among older adults—remains limited. This gap underscores the need for a deeper understanding of acetic acid's role within the complex environment of the human body, as well as the contribution of the gut microbiome, especially in the context of aging. Microbial taxa within the gut interact to form sub-communities, which often exhibit co-abundance patterns indicative of mutualistic relationships, driven by nutrient exchange and environmental modulation. For instance, certain gut bacteria produce SCFAs, which can support the growth and activity of other microbes through mechanisms like cross-feeding [34,35]. Additionally, SCFA production alters the gut environment—for example, by lowering pH—thereby shaping the microbial composition [36,37]. To unravel these complex ecological interactions and their implications for host metabolism, co-abundance

analysis offers a robust analytical framework. This approach identifies correlations among microbial taxa, including phylogenetically diverse groups that exist as cohesive ecological units which may contribute similarly to ecosystem processes. Importantly, co-abundance analysis also minimizes the impact of spurious correlations by focusing on reproducible patterns rather than isolated taxa that may be more vulnerable to noise. Against this background, this cross-sectional study aimed to investigate the relationships between the gut microbiome co-abundance patterns, plasma acetic acid, blood lipid profile, body fat mass, brain gray matter volume, and cognitive performance in a cohort of community-dwelling older adults. Additionally, the present study employed a mediation analysis to examine the role of acetic acid as a mediator in the relationship between the gut microbiome co-abundance patterns and the aforementioned health outcomes. We demonstrate that a specific gut microbial group is associated with plasma acetic acid levels, which in turn are linked to lower triglyceride levels, higher high-density lipoprotein cholesterol, reduced body fat, greater thalamic volume, and improved cognitive function in certain domains among older adults. Furthermore, plasma acetic acid mediates these microbiome–health relationships, suggesting it may be a pivotal factor connecting gut bacteria to both metabolic and neurological health.

Methods

Participants

The sample was drawn from phase 1 and phase 2 of the Integrating Systemic Data of Geriatric Medicine to Explore the Solution for Health Aging study, conducted between September 2019 and May 2023 (Figure 1). This is a prospectively enrolled observational cohort study aiming to establish a comprehensive database of geriatric medicine for Taiwanese without major physical or mental disabilities. Community-dwelling adults residing in the Songshan District, Taipei City, Taiwan or Chang Gung Health and Culture

Village, Taoyuan City, Taiwan, were recruited when undergoing health examinations at Chang Gung Memorial Hospital. In addition, participants were also recruited from the Suang-Lien, Yong-He Catholic Church and St. Theresa Senior Community Centers, which were located in northern Taiwan. Inclusion criteria were (1) age 60 years or older; (2) at least one visit to Chang Gung Memorial Hospital within 1 year of recruitment; and (3) remained in Taiwan for more than 180 days within 1 year of recruitment. To focus on healthy aging trajectories, participants with conditions that could confound physiological, metabolic, psychological, or cognitive assessments were excluded. Specifically, individuals were excluded if they exhibited clinical evidence of major organ system abnormalities, had a history of severe autoimmune disease, or were undergoing cancer treatment at the time of recruitment. These conditions are known to affect systemic inflammation and a broad range of biomarkers. Participants who had received antibiotic treatment within one month prior to enrollment were also excluded, due to the well-documented disruptive impact of antibiotics on gut microbiota composition—particularly the reduction in overall microbial diversity, the loss of key taxa, and the resulting metabolic shifts and increased susceptibility to colonization, which can lead to the development of antibiotic-resistant bacteria [79,80]. To further reduce confounding effects from cognitive or psychological impairments, exclusion criteria included an Ascertain Dementia 8 score ≥2, a Mini-Mental State Examination score ≤26, a Geriatric Depression Scale score ≥5, or a prior clinical diagnosis of dementia or major depressive disorder. Additionally, individuals who were unable to meaningfully participate in interviews due to significant hearing, visual, or cognitive impairments, or those too frail to stand and walk independently, were excluded. Frailty and mobility limitations are associated with increased risks of cognitive decline, hospitalization, and mortality [81,82], which could obscure associations intended to reflect aging processes. Including frail individuals could introduce heterogeneity not attributable to the aging trajectory itself but to underlying pathological conditions.

The participants' demographics, medical histories, and physical examinations including anthropometric characteristics were recorded. Medical records were reviewed to validate the medical history obtained through interviews. All participants were phlebotomized for general blood chemistry tests and metabolomic study.

This study was registered as an observational study at ClinicalTrials.gov (Identifier: NCT04207502).

Plasma sample preparation

Fasting blood samples were collected in EDTA-plasma vacuette tubes, with fasting defined as no food or drink except plain water for at least 8 hours prior to blood draw. Blood samples were centrifuged at 3,000 *g* at 4 °C for 10 min. Plasma supernatants were aliquoted and stored at −80 °C until analysis.

Metabolite profiling

Participants were profiled for plasma metabolites using a high-throughput proton nuclear magnetic resonance (NMR) spectroscopy. A total of 100 μL plasma sample was mixed with Bruker plasma buffer (75 mM pH 7.4 sodium phosphate) in a 1:1 ratio and the resulting 200 μL mixture was transferred into a 3 mm × 4 inch Bruker SampleJet NMR tube. All NMR analyses were performed on a Bruker Avance III HD 600 MHz spectrometer (Bruker Biospin GmbH, Rheinstetten, Germany) equipped with triple resonance probes and a Bruker SampleJet robot cooling system set at 6 °C. For each plasma sample, two automated experiments were conducted: first, a ^{1}H 1D experiment with solvent presaturation (64 scans, 98,304 data points, spectral width of 18028.85 Hz); second, a 1D Carr–Purcell–Meiboom–Gill spin–echo experiment (64 scans, 73,728 data points, spectral width of 12019.23 Hz) at 310 K. All data were processed automatically using Bruker Topspin 3.6.2 and IconNMR software (Bruker Biospin GmbH) for phasing, baseline correction, and calibration (trimethylsilylpropanoic acid to 0 ppm).

Lipoprotein reports containing 112 lipoprotein parameters for each sample were generated using the Bruker IVDr Lipoprotein Subclass Analysis method. This process involved mathematically interrogating

and quantifying the $-CH_2$ (δ = 1.25 ppm) and $-CH_3$ (δ = 0.8 ppm) peaks of the 1D spectrum after normalization to the Bruker QuantRef manager within Topspin using a PLS-2 regression model. Additionally, the automatic identification and quantification of 38 plasma metabolites were uploaded and analyzed using Bruker IVDr in plasma/serum B.I.Quant-PS.

Gut microbiome sequencing and profiling

Participants in the microbiome study collected stool samples at home using sterile, leak-proof containers and shipped them to Chang Gung Memorial Hospital. Samples were transported under ambient temperature conditions and arrived at the laboratory within 24–48 hours of collection. Upon receipt, all specimens were immediately stored at −80°C until further processing. The total genomic DNA was extracted from the samples using a column-based method (iCatcher Stool DNA Kit, CatchGene, Taipei, Taiwan) and standardized to a concentration of 1ng/μl. Full-length 16S genes (V1-V9 regions) were amplified using barcoded 16S gene specific primers (Forward: 5'Phos/GCATC-16-base barcode-AGRGTTYGATYMTGGCTCAG-3', Reverse: 5'Phos/GCATC-16-base barcode-RGYTACCTTGTTACGACTT-3') (Pacific Biosciences of California, Menlo Park, CA, USA) and sequenced using the PacBio platform following the Amplication of Full-Length 16S Gene with Barcoded for Multiplexed SMRTbell Library and Sequencing Procedure. PCR amplification was carried out using a high-fidelity DNA polymerase to minimize sequencing errors. Amplicon integrity and expected size were confirmed via gel electrophoresis. During library preparation, AMPure PB beads were employed for purification and size selection, effectively removing excess primers, nucleotides, salts, and enzymes. Negative (no-template) controls were included throughout the process to monitor for potential reagent or environmental contamination. Equimolar quantities of barcoded amplicons were pooled and subjected to SMRTbell library construction following the manufacturer's protocol (PacBio). Sequencing was performed on the PacBio Sequel II platform, producing high-fidelity (HiFi) circular consensus sequencing reads with an average read length of ~1,500 bp. Each sample achieved a minimum sequencing depth of

10,000 HiFi reads, providing sufficient resolution for taxonomic classification down to the species level. To enhance accuracy and reproducibility, a total of 18 samples were technically replicated by preparing aliquots from the same DNA extraction. Subsequently, the HiFi reads underwent further processing, including quality filtering, dereplication, learning the dataset-specific error model, amplicon sequence variant (ASV) inference, and chimera removal using the divisive amplicon denoising algorithm DADA2 package in R (R Project for Statistical Computing). The trimming and filtering were performed with a maximum of two expected errors per read (maxEE = 2). DADA2 algorithm resolves exact ASVs with single-nucleotide resolution from the full-length 16S rRNA gene with a near-zero error rate. The taxonomy classification of each representative sequence was performed using the feature-classifier and classify consensus-vsearch algorithm in QiME2 (version 2022.11), with reference to the NCBI 16S rRNA database.

Bioelectric impedance analysis (BIA)

Body composition measurements were obtained using a multi-frequency BIA device (Tanita MC-780MA P; Tanita Corporation, Tokyo, Japan), according to manufacturer's instruction.

Structural brain magnetic resonance imaging (MRI) volumetry

Volumetric gray matter measurements in this study were derived from automated segmentations of three-dimensional high-resolution T1-weighted MRI scans. MRI data were acquired using 3-Tesla scanners (Discovery MR750 or MR750w; GE Healthcare, Milwaukee, WI, USA). Three-dimensional T1-weighted imaging using inversion-recovery fast spoiled gradient-echo sequence (axial 3D BRAVO [Brain Volume Imaging]; GE Healthcare) with the following parameters: repetition time/echo time = 8.616/3.232 ms, inversion time = 450 ms, flip angle = 12°, acquisition matrix = 256 × 256 × 172, field-of-view = 256 mm × 256 mm × 172 mm, spatial resolution = 1 mm × 1 mm × 1 mm, and number of averages = 1. The T1-weighted images were processed and analyzed using Computational Anatomy Toolbox version 12.7 (dbm.neuro.uni-jena.de/cat/) within the Statistical Parameter Mapping 12 software

(fil.ion.ucl.ac.uk/spm/). The segmented brain regions were merged into 8 major regions, including frontal lobe, temporal lobe, parietal lobe, occipital lobe, basal ganglion, thalamus, cingulate, and insula.

To gain a more detailed understanding of thalamic structure in relation to plasma acetic acid levels, the thalamus was segmented into 25 subregions using Bayesian inference, following the methodology described by Iglesias et al [83]. This approach employs a probabilistic atlas derived from histological data and implemented within the FreeSurfer software. The segmentation yielded the following nuclei for each hemisphere: anteroventral (AV), laterodorsal (LD), lateral posterior (LP), ventral anterior (VA), ventral anterior magnocellular (VAmc), ventral lateral anterior (VLa), ventral lateral posterior (VLp), ventral posterolateral (VPL), ventromedial (VM), central medial (CeM), central lateral (CL), paracentral (Pc), centromedian (CM), parafascicular (Pf), paratenial (Pt), reuniens/medial ventral (MV-re), mediodorsal medial magnocellular (MDm), mediodorsal lateral parvocellular (MDl), lateral geniculate (LGN), medial geniculate (MGN), limitans/suprageniculate (L-SG), pulvinar anterior (PuA), pulvinar medial (PuM), pulvinar lateral (PuL), and pulvinar inferior (PuI).

To facilitate analysis, raw volumetric estimates of each nucleus were summed bilaterally and grouped into 10 functionally defined subfields: Anteroventral (AV), Lateral Geniculate (LGN), Medial Geniculate (MGN), Pulvinar-Limitans (comprising PuA, PuM, PuL, PuI, and L-SG), Laterodorsal (LD), Lateral Posterior (LP), Mediodorsal-Paratenial-Reuniens (including MDm, MDl, MV-re, Pt), Motor Hub (VA, VAmc, VLa, VLp), Sensory Hub (VPL, VM), and Intralaminar (CeM, CL, Pc, CM, Pf).

Cognitive assessment

Cognitive functions were assessed using the Consortium to Establish a Registry for Alzheimer's Disease (CERAD) neuropsychological battery. The CERAD battery comprises a series of standardized tests designed to evaluate multiple domains of cognitive function, including attention and concentration (digit span tasks, both forward and backward digit span), orientation (awareness of time, place, and

person), language (expression, comprehension, repetition, naming of both objects and body parts, as well as verbal fluency tasks), visuospatial and constructional ability (constructional praxis, direction perception, clock drawing, and drawing recall), memory (word list registration, immediate and delayed recall, recognition, and remote memory) and higher-order cognitive abilities (calculation, similarity and judgement). The battery was administered by trained personnel according to the CERAD manual to ensure consistency and accuracy.

Assessment of covariates

Information on smoking status, medical history, and current medications was assessed through interviews and questionnaires. Body mass index (BMI) was calculated as weight in kilograms divided by height in meters squared. Daily life physical activity was recorded with a wrist-worn, triaxial accelerometer (Actiwatch Spectrum Plus; Philips Respironics, Bend, OR, USA). Sedentary behavior was defined as activity counts < 145 counts per minute during wear-time, excluding sleep time. Average time spent in sedentary behavior is used as a measure for physical inactivity.

Statistical analysis

Statistical analyses were performed using R Statistics software (version 4.3.2; r-project.org/). To investigate the relationship between gut microbiome community composition and plasma acetic acid, the primary analyses focused on the associations between bacterial co-abundance groups and plasma acetic acid levels. Analysis of co-abundance groupings has been previously published [84,85]. Briefly, to identify co-abundance groupings (or factor representations) of bacterial taxa in the gut, Spearman correlation coefficients for the top 25 genera were calculated using the Compositionally Corrected by Renormalization and Permutation (CCREPE) package in R software. The resulting correlation matrix was used for principal factor analysis with varimax rotation. Factor loadings were applied to the 16S sequencing counts to compute an individual factor score for each bacterial co-abundance group. These scores were then used as independent variables in linear regression models. In additional secondary analyses, the microbiome diversity (both α- and β-diversity) was analyzed. For measures of within-person

α-diversity, observed taxa and the Shannon index were calculated at the species level to capture rare taxa. The observed taxa represent a measure of richness of taxa, while Shannon index is a function of both richness and evenness. The association between α-diversity and plasma acetic acid levels was examined using multivariable-adjusted linear regression models, adjusting for age, sex, and site of recruitment. For the β-diversity analysis, the between-person distances were calculated based on the Bray-Curtis distance metrics on compositional microbial genus-level relative abundance, and the significance of different factors on the β-diversity was examined with permutational multivariate analysis of variance (PERMANOVA) using the adonis function in 'vegan' package. For data visualization, non-metric multidimensional scaling (NMDS) ordination plots ($k = 3$) for the samples was constructed based on Bray-Curtis distance.

A summary of cognitive measure was generated based on principal component analysis (PCA) of the CERAD battery test results. The sample with missing results (1 participant) was excluded from analysis. Each assessment was first standardized as normal-inverse scores (mean [SD], 0 [1]).

Associations between plasma acetic acid levels and each variable in blood lipid profile, body composition, brain gray volume, and cognitive function measurements were investigated in separate linear regression, adjusting for covariates. P value < 0.05 was considered indicative of statistical significance. Corrections for multiple comparisons were performed using the Benjamini-Hochberg false discovery rate (FDR) method. P values that passed FDR correction are highlighted in the figures and tables. Participants with missing data were excluded from regression models on a covariate basis (1 for BMI).

To assess the robustness of BIA-derived body composition measurements, we conducted sensitivity analyses based on BMI categories as defined by the World Health Organization. BMI was classified into four ordinal categories: underweight (<18.5 kg/m²), normal weight (18.5–24.9 kg/m²), overweight (25.0–29.9 kg/m²), and obese (≥30.0 kg/m²). We employed covariate-adjusted ordinal logistic regression to examine the independent association between plasma acetic acid concentration and increasing BMI categories.

Formal mediation analyses were performed to examine the relationship between gut microbiota co-abundance group, plasma acetic acid levels, plasma triglyceride, plasma high-density lipoprotein (HDL) cholesterol, body fat mass, brain gray matter volume, and cognitive function. Mediation analyses were conducted using the mediation package in R, employing the default quasi-Bayesian approach. The total effects of the gut microbiota co-abundance group on the plasma triglyceride, plasma HDL cholesterol, body fat mass, brain gray matter volume and cognitive function were assessed. The average causal mediation effect (ACME), acting through plasma acetic acid as a mediating variable, as well as the average direct effect (ADE) not mediated by plasma acetic acid, were calculated. Proportion mediated was defined as the ratio of ACME to the total effect. Adjusted linear regression models were used for the mediation analyses.

To obtain robust estimates of uncertainty, we employed a non-parametric bootstrap procedure that encompassed the entire analytical pipeline. Specifically, the bootstrapping process included bacterial co-abundance measures followed by regression or mediation analyses, ensuring that all steps of the statistical chain contributed to the estimation of variability. Similarly, for analyses involving cognitive function, PCA was integrated within the bootstrap loop to derive confidence intervals for the resulting components and their associations. Bootstrapping was performed using the "boot" package in R with 1,000 iterations.

Results

Participant characteristics

A total of 580 participants were enrolled in the study. Five participants withdrew from the study, resulting in a final analytic sample of 575 participants (mean [standard deviation] age, 72.5 [6.4] years; 338 [58.8%] women) (Table 1). The 575 participants underwent plasma metabolomic profiling, 555 underwent BIA, 529 provided stool samples for 16S rRNA analysis, 272 underwent brain MRI, and 318

received neuropsychological tests assessment. All participants who underwent brain MRI received neuropsychological assessment.

MRI scans of three patients were excluded from the analysis (one had an extraaxial mass, one had a large choroid cyst, one had a previous large infarction). The participants who underwent MRI and those who did not differed significantly in education levels (Supplemental Table 1). Distributions for BIA measures are shown in Supplemental Table 2.

In the gut microbiome analysis, we identified 58,911 unique ASVs, with a mean of 250 ASVs per sample (range: 29–546). At the genus level, 415 unique genera were detected, with a mean of 58 genera per sample (range: 9–108). Alpha diversity at the species level, measured by the Shannon index, had a median value of 4.59 (interquartile range: 4.21–4.88). No prevalence or relative abundance filtering was applied during diversity analyses. The descriptive statistics for the genera included in the analysis of the gut microbiome are shown in Supplemental Table 3.

Gut bacterial co-abundance groupings

Based on a scree plot, three factors were extracted for factor analysis of the 25 most abundant gut bacterial genera (Figure 2 and Supplemental Table 4). The first factor captured co-abundance of Sphingomonas and Methylobacterium genera (loadings, > 0.9). For the second factor, positive loadings for Oscillibacter (loading, 0.492), and Coprococcus (loading, 0.492) were most substantial. Phocaeicola (loading, −0.581) and Bacteroides (loading, −0.453) were negatively associated with this Oscillibacter and Coprococcus taxa co-abundance grouping. The third factor was defined mainly by Mediterraneibacter (loading, 0.406) and Phascolarctobacterium (loading, 0.400), with negative factor loadings for Prevotella (loading, −0.439).

Gut bacterial co-abundance groupings and plasma acetic acid levels

The score of factor 2 (representing an increase in the abundance of Oscillibacter and Coprococcus taxa and a decrease in the abundance of Phocaeicola and Bacteroides) was associated with a higher plasma acetic acid concentration (standardized β, 0.12; bootstrap 95% confidence interval [CI], 0.04 to 0.19; FDR-corrected P = 0.01) in multivariate regression analysis (adjusted for age, sex, BMI and site of recruitment), where a 1 unit increase in factor 2 was associated with approximately a 0.12 unit increase in plasma acetic acid concentration. Multivariate regression models did not detect any significant association between bacterial co-abundance grouping scores of factor 1 or 3 and plasma acetic acid concentration.

Gut microbial diversity and plasma acetic acid levels

In multivariable adjusted (age, sex, BMI, and site of recruitment) regression analysis for α-diversity, higher plasma acetic acid levels were associated higher observed taxa richness (standardized β, 0.11; 95% CI, 0.03 to 0.20; P = 0.01) but not significantly associated with Shannon index (standardized β, 0.08; 95% CI, −0.01 to 0.17; P = 0.07). The scatterplot of plasma acetic acid levels and observed taxa is displayed in Figure 3A. The β-diversity (Bray-Curtis dissimilarity matrix) of gut microbiota was significantly associated with plasma acetic acid levels (P = 0.01), after adjusting for age, sex, BMI, and site of recruitment. The NMDS ordination plot of the Bray-Curtis distance of bacterial community is shown in Figure 3B.

Plasma acetic acid levels and blood lipid profile

Results are presented in Figure 4A and Supplemental Table 5. After adjustment for clinical variables (age, sex, lipid-lowering medication, hypertension, diabetes, smoking status, alcohol drinking and physical inactivity) and FDR correction, plasma triglyceride levels were negatively associated with plasma acetic acid concentrations (standardized β, −0.12, 95% CI, −0.21 to −0.04; FDR-corrected P = 0.02). In addition, in the adjusted model, plasma HDL cholesterol levels were positively associated with plasma acetic acid

concentrations (standardized β, 0.15, 95% CI, 0.05 to 0.23; FDR-corrected P = 0.01). No significant association was noted between plasma acetic acid concentrations and plasma total cholesterol, low-density lipoprotein cholesterol, Apo-A1, Apo-A2, and Apo-B100 levels.

Plasma acetic acid levels and body composition measures

Results are presented in Figure 4B and Supplemental Table 6. Higher plasma acetic acid levels were associated with lower BMI (standardized β, −0.13; 95% CI, −0.22 to −0.04; FDR-corrected P = 0.02) and lower fat mass (standardized β, −0.14; 95% CI, −0.23 to −0.06; FDR-corrected P = 0.01), after adjusting for age, sex, hyperlipidemia, hypertension, diabetes, smoking status, physical inactivity and height (the analysis of BMI did not include height as covariate). No significant association was noted between plasma acetic acid levels and fat-free mass, muscle mass, bone mass, appendicular skeletal muscle mass, total body water, extracellular water, and intracellular water.

To confirm the robustness of the observed associations between plasma acetic acid levels and BIA-derived body composition measurements, sensitivity analyses were performed. Using World Health Organization-defined BMI categories for stratification, results demonstrated that elevated plasma acetic acid levels correlated with lower BMI categories in unadjusted ordinal regression models (odds ratio [OR] per 1-SD increase: 0.70, 95% CI, 0.58–0.83; P < 0.001). This association remained after adjustment for age, sex, hyperlipidemia, hypertension, diabetes, smoking status, and physical inactivity (adjusted OR: 0.73, 95% CI, 0.59–0.89; P = 0.002). The proportional odds assumption was validated using the Brant test (P = 0.24).

Plasma acetic acid levels and gray matter volumes

The volume of thalamus was significantly associated with plasma acetic acid levels (standardized β, 0.15; 95% CI, 0.05 to 0.26; FDR-corrected P = 0.04), after adjusting for age, sex and total intracranial volume. The volumes of basal ganglia, temporal lobes, parietal lobes, frontal lobes, occipital lobes, cingulate gyri,

and insulas were not significantly associated with plasma acetic acid levels (Figure 4C, Supplemental Table 7). In the analysis of thalamic subfields, none of the ten evaluated subfields demonstrated a statistically significant association with plasma acetic acid levels after adjustment for age, sex and intracranial volume (Supplemental Table 8).

Plasma acetic acid levels and cognitive functions

Based on scree plot, four principal components (PC1 to PC4) of the cognitive functions were extracted, which explained a cumulative variation of 47%. PC1 represented learning and memory, with high loadings of the scores of word list registration, recall, and recognition. PC2 represented language functions, with high loadings of the scores of naming objects and body parts. PC3 represented visuospatial constructional ability and calculation, while PC4 represented judgement (Figure 5).

In the adjusted analysis controlling for age, sex and educational levels, plasma acetic acid levels were positively associated with the PC4 of cognitive functions (Figure 4D, standardized β, 0.18; 95% CI, 0.12 to 0.34; FDR-corrected P = 0.004). No significant association was observed between plasma acetic acid levels and PC1, PC2 or PC3 of cognitive functions (Figure 4D, Supplemental Table 9). A summary of the cognitive domains evaluated in relation to thalamic volume and executive function is presented in Supplemental Table 10.

Mediation analysis

Figure 6 and Supplemental Table 11 document the estimates of ACME, ADE, total effect, and proportion mediated for the association of the gut bacterial co-abundance group (factor 2) with plasma triglyceride, plasma HDL cholesterol, body fat mass, thalamic volume, and PC4 cognitive performance. Negative ACMEs of the gut microbial co-abundance group on plasma triglyceride and body fat mass via plasma acetic acid were observed (standardized β, −0.01; 95% CI, –0.03 to –0.00, P = 0.003 and standardized β,

−0.01, 95% CI, −0.04 to −0.00, *P* = 0.008, respectively). Positive ACMEs on plasma HDL cholesterol, thalamic volume and PC4 cognitive performance via plasma acetic acid were also noted (standardized *β,* 0.02; 95% CI, 0.01 to 0.05; *P* = 0.001 and standardized *β*, 0.02; 95% CI, 0.01 to 0.07; *P* = 0.005; standardized *β*, 0.03; 95% CI, 0.02 to 0.07; *P* = 0.001, respectively). The gut microbial co-abundance group (factor 2) had significant total effects and ADEs on plasma triglyceride (standardized *β,* −0.13; 95% CI, −0.28 to −0.02; *P* = 0.03 and standardized *β*, −0.12; 95% CI, −0.26 to −0.01; *P* = 0.04, respectively), while the total effects and ADEs on plasma HDL cholesterol, body fat mass, thalamic volume and PC4 cognitive performance were not significant.

To further explore the directionality of the observed associations, we conducted a reverse mediation analysis, treating plasma acetate as the exposure and gut microbial co-abundance group 2 as the potential mediator, with health outcomes including plasma triglyceride levels, HDL cholesterol levels, fat mass, thalamic volume, and PC4 cognitive performance. Among these models, only the one predicting plasma triglyceride levels showed a statistically significant ACME for co-abundance group 2 (standardized *β* = –0.02, 95% CI: –0.06 to –0.00, *P* = 0.01) (Supplemental Table 12).

Discussion

This cross-sectional study of a community-dwelling older cohort found associations between plasma acetic acid levels and blood lipid profile, body composition and brain gray matter volume, independent of demographic factor, medical history, medication, and lifestyle factors. Specifically, we observed that higher plasma acetic acid levels were associated lower plasma triglyceride, higher blood HDL cholesterol, lower BMI, lower body fat mass, and higher thalamic volume. Gut microbial diversity, both *α* diversity and *β* diversity, was significantly associated with plasma acetic acid levels. In addition, our findings

suggest a potential mediating role of plasma acetic acid in the relationship between gut microbiome Oscillibacter and Coprococcus-dominated co-abundance grouping, blood lipid profile, body fat mass, thalamus volume and cognitive function. Coprococcus species are recognized acetate producers and have been reported to decline with age [13,38]. This reduction may reflect a diminished microbial capacity for acetate production in older adults. The association between factor 2 and plasma acetate levels supports its biological relevance and highlights the potential impact of age-related microbiota shifts on host metabolism. Notably, the total effects of gut microbiome co-abundance grouping on plasma HDL cholesterol, body fat mass, thalamic volume and cognitive function were not significant, which may imply the existence of multiple mediators acting in different directions. It is also worth noting that the measurement tools used in this study, such as BIA for body fat estimation and the CERAD battery for cognitive assessment, may lack the sensitivity to detect subtle associations. Although widely used and practical, their limited accuracy can obscure nuanced relationships. For instance, BIA may underestimate body fat in obese individuals due to assumptions about hydration and body geometry based on normal-weight populations [39]. Similarly, while the CERAD battery effectively identifies broad cognitive deficits, has notable limitations in overall sensitivity and domain coverage [40,41]. Therefore, our findings should be interpreted with caution, and future studies may benefit from using more sensitive or comprehensive tools. Overall, this cross-sectional study suggests that plasma acetic acid may serve as a key mediator linking gut microbiota with blood lipid profiles, adiposity, brain volume, and cognitive performance in older adults, representing a potential geroprotective approach. While our mediation analysis provides insights into potential pathways, we acknowledge that such analysis relies on several assumptions for a valid causal interpretation, particularly the assumption of sequential ignorability. These assumptions include the absence of unmeasured confounding in the relationships between the exposure, mediator, and outcome, as well as the correct specification of the underlying models. Given the observational design of our study, these assumptions may not be fully satisfied. Therefore, our results should be interpreted with caution and regarded as exploratory rather than definitive evidence of causal mediation. To strengthen generalizability

and confirm the robustness of these findings, future research should prioritize replication in independent cohorts. Longitudinal or interventional studies evaluating comparable endpoints would provide critical validation of these associations while clarifying causal relationships and temporal dynamics.

Although our study showed potential mediating role of plasma acetic acid levels in the association between the gut microbiome and various health outcomes, contrary to our expectations, we did not observe statistically significant total effects of the gut bacterial co-abundance group on serum HDL cholesterol, body fat mass, thalamic volume, or PC4 cognitive performance. Several factors may account for these null findings. First, the relatively modest sample size may have limited the statistical power of our analyses, reducing our ability to detect subtle associations with small effect sizes. Additionally, unmeasured confounding variables—such as plasma levels of propionic acid and butyric acid—may have interacted with plasma acetic acid, either synergistically or antagonistically, thereby complicating the overall impact of bacterial co-abundance on health outcomes. Furthermore, other potential mediators, such as cardiometabolic factors and systemic inflammatory markers, that could help explain the observed relationships were not included in the present study. Another important consideration is the geographic and demographic specificity of our cohort, comprising older Taiwanese adults, which may have contributed to the non-significant total effects observed in our analyses. Gut microbiota composition is known to be influenced by age, lifestyle, and regional environmental exposures. In older adults, microbial diversity often declines, which may have limited the variability necessary to detect significant microbiome-related associations. Additionally, region-specific environmental exposures and genetic predispositions unique to this Taiwanese cohort, which may either enhance or attenuate observable effects, were not accounted for in our analysis. Future research should incorporate these variables to validate and refine the proposed pathways, particularly through the use of controlled interventional study designs.

Among the reverse mediation models with plasma acetate as the exposure and gut microbial co-abundance group 2 as the potential mediator, only the model predicting plasma triglycerides levels showed

a statistically significant ACME of this co-abundance group. This finding implies a potentially bidirectional relationship between acetate and co-abundance group 2 in modulating plasma triglyceride levels, possibly reflecting complex metabolic feedback mechanisms. In contrast, no significant ACME was observed in the models for plasma HDL cholesterol levels, fat mass, thalamic volume, or PC4 cognitive performance, indicating that this microbial group is less likely to mediate the influence of acetate on those outcomes. The absence of significant mediation in the other outcomes supports the directionality proposed in our original model and strengthens the validity of the observed associations.

Human experimental studies involving the colonic administration of ^{13}C-labeled SCFAs via colon delivery capsules have confirmed that acetate enters systemic circulation and is incorporated into cholesterol and fatty acids [42]. On top of these findings, our study revealed associations between acetate levels and plasma HDL cholesterol and triglycerides, alongside a correlation between elevated acetate and reduced body fat mass. These findings suggest that acetate may function not only as a metabolic substrate but also as a regulator of metabolic pathways. This notion is supported by several animal and *in vitro* studies. The consumption of acetic acid in rodents has been shown to reduce accumulation of abdominal and liver fat [43]. In addition, subcutaneous acetate administration in rabbits inhibits lipid accumulation via multiple signaling pathways, such as AMPK and ERK1/2 in the liver, PPARα in skeletal muscle, and GPR41/43, AMPK, mTOR, and ERK1/2 in adipose tissue [44]. Experimental *in vitro* and rodent studies investigating the acetate-related 5′AMP- AMPK phosphorylation signaling pathway have shown that its activation can promote the downstream secretion of leptin by adipocytes, as well as peptide YY and glucagon-like peptide-1 by enteroendocrine cells, thereby exerting an anorexigenic effect on the host [45-47]. Nevertheless, contradictory results have also been reported, showing that increased acetate production by altered gut microbiota triggers the parasympathetic nervous system, resulting in higher insulin and ghrelin secretion, increased appetite, obesity, and associated ramifications [48]. Several human studies have been conducted with a relatively small number of participants to investigate the impact of

acetic acid supplementation on lipid profiles and weight loss [49-51]. While disparities persist—potentially due to variations in dosage, metabolic context, or microbiota composition—our findings underscore acetate's critical role in linking microbial metabolism to host energy homeostasis. This relationship highlights its therapeutic promise, even amidst these unresolved complexities.

A recent analysis of fecal metagenomics and metabolomics in the Framingham Heart Study revealed that individuals with high α-diversity and a prevalence of Oscillibacter exhibited lower levels of plasma triglycerides and glucose, along with higher levels of plasma HDL cholesterol [52]. This association was believed to be linked to the cholesterol-metabolizing abilities identified in *Oscillibacter* spp. Consistent with these findings, our data showed that a higher bacterial co-abundance score that represents increased Oscillibacter taxa was associated with lower plasma triglycerides and higher blood HDL cholesterol levels. Moreover, we found that this relationship was mediated by plasma acetic acid levels, a factor not emphasized in previous studies. This potential pathway, through which gut microbiota might influence lipid metabolism, suggests new targets for therapeutic intervention in dyslipidemia and related metabolic disorders.

Recent evidence suggests that acetate, may influence brain volume and cognitive processes through multiple mechanisms. Rodent studies have demonstrated that acetate can cross the blood-brain barrier (BBB), as evidenced by the detection of ^{14}C-labeled SCFAs in brain tissue following carotid artery injection [53]. In human, according to the Human Metabolome Database (http://www.hmdb.ca/), acetate concentrations in cerebrospinal fluid range from 0 to 171 μM. In an animal study, acetate administration in mice was shown to enhance brain plasticity and increase brain-derived neurotrophic factor mRNA expression in the hippocampus [54]. However, in our study, hippocampal volume was not significantly associated with plasma acetic acid levels. In addition, acetate administration has been reported to increase cerebral blood flow in the thalamus in both healthy individuals and those with alcohol use disorder [55,56], aligning with our findings that link higher plasma acetic acid levels with increased thalamic volume. In

the central nervous system, acetate is known to modulate orexin/hypocretin neurons in hypothalamus [57]. Furthermore, acetate's neurobiological effects may be mediated indirectly through systemic or epigenetic pathways as well as direct receptor signaling. Emerging data also indicate that SCFAs help maintain BBB integrity[56], support microglial maturation[58], and modulate synaptic plasticity[59], highlighting their potential role in neuroprotection and cognitive regulation. Moreover, despite their relatively low concentrations in the brain, SCFAs—particularly acetate—actively participate in central metabolic processes [60]. While these findings suggest a role for acetate in brain physiology, the existing literature linking SCFAs to specific brain volumes is limited, and our study contributes to this emerging field of research.

Preclinical studies have demonstrated that SCFAs, particularly acetate and butyrate, exert neuroprotective effects across various models of cognitive impairment. In sleep-fragmented mice, acetate supplementation mitigates metabolic dysfunction and cognitive deficits [61]. In aged murine models, acetate attenuates perioperative neurocognitive disorders [62], while in rodent models of Alzheimer's disease, butyrate improves memory via histone deacetylase inhibition [63], and acetate reduces neuroinflammation through GPR41-mediated suppression of pro-inflammatory signaling [64]. Conversely, disruption of the gut microbiome, such as through vancomycin exposure or depletion of acetate-producing bacteria, has been associated with cognitive impairment in diabetic mice [33]. In models of vascular dementia, *Clostridium butyricum* administration improved cognitive outcomes, and prebiotic supplementation in rats elevated plasma acetate and N-methyl-D-aspartate receptor expression, enhancing cognitive flexibility [65]. While these findings underscore the potential of SCFAs to modulate cognition—most of the existing research involves animal studies assessing memory and spatial learning tasks—human studies remain limited, particularly those examining higher-order executive functions, thereby limiting comparability. Our study helps bridge this translational gap and highlights SCFAs as promising therapeutic targets for cognitive health.

Notably, brain volume atrophy and cognitive decline in older adults are generally considered to be gradual, progressive processes influenced by a complex interplay of multiple factors rather than a single mechanism. While acetic acid has been proposed to exert effects on the central nervous system through pathways involving BBB integrity and immune modulation, these mechanisms are likely influenced by other gut-derived metabolites (such as methylamine trimethylamine N-oxide) [66]. This complexity may obscure the isolated contribution of acetic acid to brain structure and cognitive function. Moreover, the gut microbiome itself is a dynamic ecosystem that changes over time, particularly with aging. As such, a single cross-sectional assessment may not accurately capture the temporal variability in microbial composition and its cumulative effects on long-term neurological outcomes. These considerations may render the clinical applicability of acetic acid as a therapeutic target less consistent for brain and neurological outcomes compared to other domains, such as body composition or lipid profile.

Judgement is the ability to evaluate situations and make informed decisions by carefully considering relevant circumstances, contextual factor, potential solutions and consequences [67]. From a neuropsychological standpoint, judgement encompasses various cognitive components, such as memory, sustained attention and reasoning [68] but especially engages the executive functions [69]. The prefrontal cortex is recognized as an important brain structure for executive function [70-73]. Patients suffered from prefrontal cortex damage demonstrate impaired judgement, planning, and decision-making abilities [71,73]. To integrate and coordinate information, the prefrontal cortex is densely connected to several brain structures, one of which is the thalamus [73]. Recent study showed that the thalamus regulates subnetworks within the frontal cortex, which encode distinct aspects of decision making [74]. In the present study, we found that acetic acid mediates the relationship between the gut microbiome, thalamus volume, and cognitive performance related to judgement. However, it remains unclear whether this mediation of cognitive function occurs through its effect on the thalamus. While this plausible based on neuroanatomical knowledge and recent neuroscience research, further studies are warranted to explore this point.

The observed association between total thalamic volume and plasma acetic acid levels, in the absence of significant associations with individual thalamic subfields, may reflect a cumulative or distributed effect across multiple subregions, each contributing modestly to the overall relationship. When analyzed individually, the effects within each subfield may be too subtle to reach statistical significance, particularly after correction for multiple comparisons. Furthermore, methodological constraints inherent to thalamic subfield segmentation warrant consideration. The limited intrinsic contrast of thalamic nuclei on conventional T1-weighted MRI, combined with their small volumetric dimensions, introduces measurement variability. Such technical challenges may reduce the statistical power to identify subtle subfield-specific effects, even if present. Consequently, the observed whole-thalamus association might reflect a more robust biological signal that persists despite measurement noise, whereas subfield-level analyses remain constrained by current imaging limitations.

Traditionally, the use of probiotics has been advocated as a therapeutic strategy in various diseases, with the assumption that introducing beneficial bacteria into the gut can alter gut microbiota composition and alleviate effect of microbial dysbiosis. However, our findings suggest that targeting acetic acid—the mediator in this relationship—may provide a more direct intervention. Unlike probiotics, which rely on the survival and competitive advantage of specific bacteria within the intestinal environment, modulating acetic acid could bypass the challenges of bacterial competition, potentially offering a more reliable and sustained therapeutic effect. Moreover, acetic acid, long used as a food flavoring, has been proven safe for human consumption. This shift in perspective opens new avenues for intervention strategies, highlighting acetic acid as a promising target for therapeutic development in place of conventional probiotic supplementation in metabolic and neurologic disorders. Nevertheless, further longitudinal and interventional studies, as well as animal experiments are warranted to confirm these observational findings in our study.

While 16S rRNA sequencing has long been a cornerstone of microbiome research due to its efficiency in profiling gut microbial communities, this method primarily focuses on taxonomic identification and offers limited insight into the functional dynamics and metabolic potential of the microbiome. To address this limitation, future studies should consider incorporating functional microbiome profiling techniques such as shotgun metagenomics [75,76]. These approaches allow for a comprehensive analysis of microbial genes and pathways, providing deeper insights into the functional roles and metabolic capabilities of microbial communities. Such advancements would enhance our understanding of the mechanistic roles that microbial communities, particularly those involved in acetate production.

There are several limitations in this study that need to be addressed. First, the cross-sectional study design prevents the evaluation of temporality and causality. Second, since plasma propionate and butyrate were not detected using nuclear magnetic resonance spectroscopy in this study, we were unable to adjust for their potential confounding effects in the analysis. Both propionate and butyrate are important gut microbiota-derived SCFAs that signal through shared G-protein-coupled receptors and are involved in overlapping metabolic pathways. Moreover, the interconversion among acetate, propionate, and butyrate by gut microbes further underscores their interconnected biological significance. The inability to account for propionate and butyrate in our analysis limits our capacity to fully adjust for potential confounding effects, representing an important gap in our assessment of gut microbiota-derived metabolites. Third, it is crucial to recognize that dietary habits exert a profound influence on the composition of gut microbiota [77]. Variations in dietary patterns may have contributed to inter-individual differences in microbial profiles, which, in turn, affect SCFA levels. In addition, dietary fiber is fermented by gut bacteria to produce SCFA; thus, increased fiber intake may enhance SCFA production. Therefore, future studies should incorporate comprehensive dietary assessments, including detailed records of food frequency and portion size, to more accurately account for this pivotal modulatory factor. Forth, since the composition of the intestinal

microbiome varies by location within the intestinal tract, the use of stool samples alone may not provide a complete characterization; sampling from different intestinal sites could offer a more comprehensive understanding. Fifth, other factors that could be linked to the composition of the gut microbiota, such as antibiotic use and colonic transit time, were not taken into account in this study. Sixth, the absence of stool consistency data represents a notable limitation. Given its strong associations with species richness, enterotypes, and community composition [78], assessing stool consistency—e.g., via the Bristol Stool Scale—is essential in microbiota studies. Future research should include this parameter to enable proper confounder analysis and strengthen the robustness of microbiome findings. Seventh, despite adjusting for several covariates, it was not possible to rule out the potential influence of residual confounders. The gut microbiota exert their effects on health through a variety of mechanisms, including humoral signaling and neural pathways, many of which are difficult to fully quantify or control for in observational studies. These mechanisms are also modulated by external factors—such as diet, medication use, and lifestyle—which independently influence host metabolism, immune function, and overall health. Consequently, these factors may introduce confounding pathways that affect both gut microbiota composition and health outcomes. Therefore, unmeasured or inadequately measured variables may have contributed to the observed associations, and caution is warranted when interpreting these findings or drawing causal inferences. Eighth, although our study cohort included participants from multiple sites across northern Taiwan, it primarily represents the aging population within this specific geographic region. This regional constraint may limit the broader applicability of our findings to aging populations in other parts of Taiwan or globally, where demographics, genetic background, dietary habits, and gut microbiota compositions may differ. Furthermore, recruitment occurred at community centers and during routine health examinations, which tends to attract individuals with more active lifestyles and greater health consciousness. This introduces a degree of selection bias, as such participants may not fully represent the general aging population, particularly those with limited mobility, lower health awareness, or reduced access to preventive healthcare services. Additionally, we observed a significant difference in educational

attainment between participants who underwent MRI scans and those who did not. As higher education levels are often linked to increased health literacy and engagement with healthcare, this may further skew the data and contribute to selection bias. Collectively, these factors may affect the generalizability of our findings and should be considered when interpreting the study's implications. While our results may be applicable to populations with similar age ranges and racial or ethnic backgrounds, further research involving more diverse populations is warranted.

In conclusion, our results suggest that plasma acetic acid mediates the relationship between the gut microbiome, blood lipid profile, body fat mass, thalamus volume, and cognitive performance in older adults. Increased co-abundance of Oscillibacter and Coprococcus was associated with higher plasma acetic acid levels. This research could offer new insights into the causes, mechanisms, prevention, and treatment of dyslipidemia, related metabolic disorders, as well as neurological diseases in older adults, highlighting the potential of acetic acid as an intervention target for diseases.

Study approval

This cohort study was approved by the institutional review board of Chang Gung Medical Foundation, Taiwan (approval number: 201900702A3 and 202100554B0). All research procedures adhered to the tenets of the Declaration of Helsinki. Informed consent was obtained from all participants.

Data availability.

The data that support the findings of this study are available on request from the corresponding author C.H.T.. A decision regarding data access will be provided within four weeks of receipt of a complete

request. The data are not publicly available because they contain information that could compromise research participant privacy or consent.

Code availability

The code used for data analysis in this study is not publicly available but can be provided by the corresponding author upon reasonable request.

Author contributions

TYS and CHT conceived and designed the study. TYS, CHT, JTF, CHC, YMY, MLC, CJL, AMCW, SNL, and CPL acquired, analyzed, or interpreted data. TYS and CHT drafted the manuscript. All authors critically revised the manuscript for important intellectual content. TYS and CHT performed statistical analysis. JTF obtained funding.

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Conflict of Interest Statement

The authors declare that they have no conflicts of interest.

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Table 1 Participant characteristics for individuals who received plasma metabolomic profiling: Integrating Systematic Data of Geriatric Medicine to Explore the Solution for Health Aging study

Variable	**Total (N = 575)**
Demographic Characteristic	
Age, mean (SD), y	72.5 (6.4)
Female, No. (%)	338 (58.8)
Education, No. (%)	
< High school degree	60 (10.4)
High school degree	117 (20.3)
Junior college degree	104 (18.1)
College degree	288 (50.1)

Missing data	6 (1.0)
Health Characteristic	
Current smoker, No. (%)	15 (2.6)
Blood pressure, mean (SD), mmHg	
Systolic	135.7 (18.2)
Diastolic	75.6 (11.6)
Heart rate, mean (SD), bpm	75.7 (11.1)
Body mass index, mean (SD)	23.7 (3.3)
Waist circumference, mean (SD), cm	83.6 (8.9)
Lipid level, mean (SD), mg/dL	
Total cholesterol	188.8 (36.9)
LDL cholesterol	106.0 (30.5)
HDL cholesterol	60.8 (13.6)
Triglycerides	92.7 (44.3)
Lipid-lowering medication, No. (%)	115 (20.0)
History of diabetes, No. (%)	103 (17.9)
History of hypertension, No. (%)	244 (42.4)

Abbreviations: SD, standard deviation; LDL, low-density lipoprotein; HDL, high-density lipoprotein

Unless otherwise indicated, data are expressed as number (percentage) of participants.

Figure Captions

Figure 1 Study design and included participants

Figure illustrating participant inclusion and exclusion criteria. A total of 580 participants met the eligibility criteria, with 5 excluded due to early withdrawal. The final number of included participants is shown, and subcohorts used for specific analyses are indicated.

Figure 2 Co-abundance analysis of gut microbiome

A) Spearman correlation matrix of the top 25 genera. The correlation matrix was subjected to principal factor analysis. **B)** Factor loadings were applied to the 16S sequencing counts to derive individual factor scores for each bacterial co-abundance group. A total of three co-abundance factors were extracted.

Figure 3 Gut microbial diversity and plasma acetic acid levels

A) Scatterplot of plasma acetic acid levels vs observed taxa (richness) of the gut microbiome at species level (n = 529). The solid line represents least-squares fit, shaded area is the 95% confidence interval bounds of the slope, and dashed blue lines are the 95% prediction interval for new observations. **B)** Plot of the first two dimensions of the NMDS ordination based on Bray-Curtis distances of relative abundance at the genus level was used to visualize differences in gut microbial community composition between samples with increasing plasma acetic acid levels. Fitted contour lines were added to document plasma acetic acid levels. Abbreviations: NMDS, non-metric multidimensional scaling.

Figure 4 Associations between plasma acetic acid levels and clinical measures

Associations between plasma acetic acid levels and blood lipid profile **(A)**, body composition **(B)**, gray matter volume **(C)**, and cognitive function **(D)**. Solid dots and error bars represent the beta coefficient and the 95% confidence intervals respectively. Two-tailed *t*-tests were used to assess the statistical significance of beta coefficients. Uncorrected *P* values are presented, and coefficients that remained significant after false discovery rate correction are indicated. Statistical significance is indicated by: * $p <$ 0.05; † false discovery corrected $p < 0.05$

(*n* = 522 (A), 511 (B), 269 (C), and 318 (D) biologically independent subjects.)

Figure 5 Principal component analysis of cognitive measures

Heatmap of the loadings for all principal components of cognitive measures was shown on the left. Four components were extracted, as shown on the right, representing learning/memory (component 1), language (component 2), visuospatial ability/calculation (component 3), and judgement (component 4). All weightings of at least 0.30 were depicted. Abbreviations: PC, principal component; CERAD, Consortium to Establish a Registry for Alzheimer's Disease.

Figure 6 Scatter plots, mediation analysis diagrams and effect decomposition plots

The bacterial co-abundance factor 2 score and plasma acetic acid levels were modelled as the exposures and mediators respectively in each model. The serum triglyceride levels **(A)**, serum high-density lipoprotein cholesterol **(B)**, body fat mass **(C)**, thalamus volume **(D)**, and PC4 cognitive performance **(E)** were modelled as outcomes. The scatter plots show the relationship between plasma acetic acid levels and various health outcomes. The solid lines in the scatter plots represent the least-squares fit, the shaded areas indicate the 95% confidence interval of the slope, and the dashed blue lines show the 95% prediction interval for new observations. Solid dots and error bars in the effect decomposition plots represent point estimates and 95% confidence interval. Abbreviations: ACME, average causal mediation effect; ADE, average direct effect; HDL, high-density lipoprotein.

(*n* = 529 (A), 529 (B), 518 (C), 269 (D), and 318 (E) biologically independent subjects.)

ED Summary:

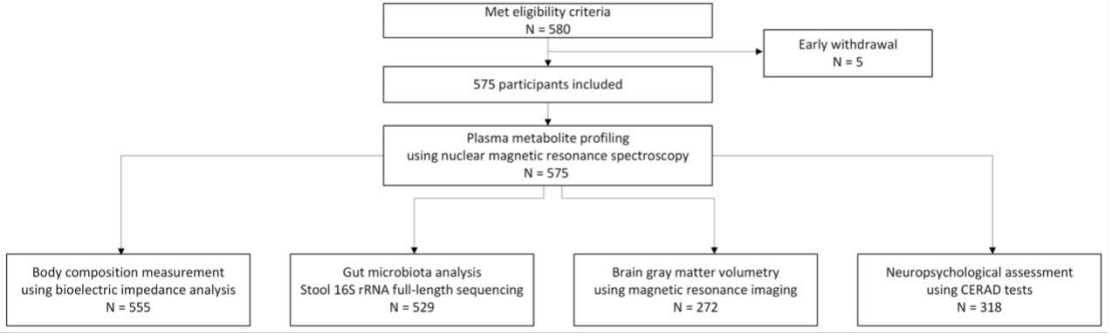
Siow et al. examine how gut microbiota relate to plasma acetic acid and whether acetic acid mediates associations with health outcomes in older adults. The results show that higher acetic acid levels link to lower

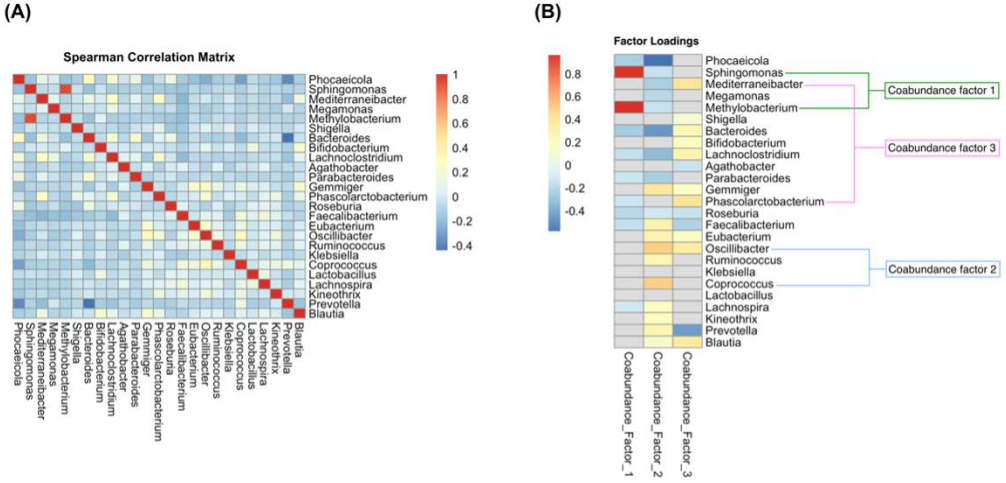
triglycerides, higher HDL, lower BMI and body fat, larger thalamic volume, and better performance in select cognitive domains.

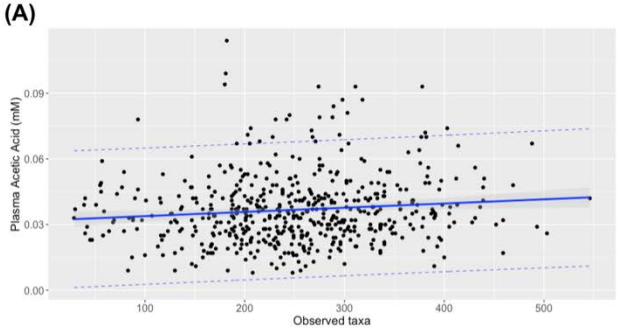
Peer review information: *Communications Medicine* thanks Theis Lange and Anja Lok for their contribution to the peer review of this work.

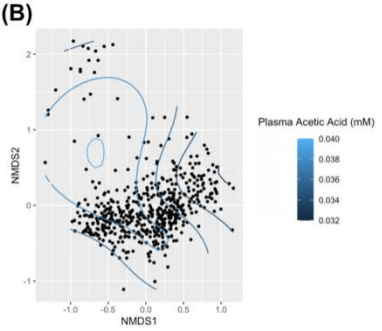
Inclusion Criteria
Age 60 years or older At least one visit to Chang Gung Memorial Hospital within 1 year of recruitment Remained in Taiwan for more than 180 days within 1 year of recruitment

Exclusion Criteria
Clinical evidence of major organ system abnormalities History of severe autoimmune disorders Cancer treatment at recruitment Antibiotic treatment within 1 month of recruitment Ascertain Dementia 8 score of 2 or above, Mini-Mental State Examination score of 26 or less, or Geriatric Depression Scale score of 5 or above Outpatient follow-up for cognitive problems Physician-diagnosed dementia or major depressive disorder Significant hearing, visual, or cognitive impairments or inability to participate in interviews in a meaningful manner Too frail to stand and walk





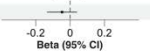




(A) Blood Lipid Profile - Plasma Acetic Acid Levels

Variable	Beta	(95% CI)	Uncorrected *P*-value
Triglyceride	-0.13	(-0.21 to -0.04)	0.005 *,†
Total Cholesterol	0.02	(-0.06 to 0.10)	0.568
HDL Cholesterol	0.13	(0.05 to 0.21)	0.002 *,†
LDL Cholesterol	0.01	(-0.07 to 0.09)	0.847
Apo-A1	0.08	(0.00 to 0.16)	0.046 *
Apo-A2	0.00	(-0.08 to 0.07)	0.923
Apo-B100	-0.05	(-0.13 to 0.04)	0.285

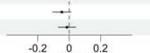
*Association had uncorrected P < 0.05
†Association had FDR-corrected P < 0.05



(B) Body Composition - Plasma Acetic Acid Levels

Variable	Beta	(95% CI)	Uncorrected *P*-value
BMI	-0.13	(-0.22 to -0.04)	0.004 *,†
Fat mass	-0.14	(-0.23 to -0.06)	0.001 *,†
Fat-free mass	-0.03	(-0.07 to 0.00)	0.073
Muscle mass	-0.03	(-0.07 to 0.00)	0.075
Bone Mass	-0.05	(-0.10 to 0.00)	0.075
ASM	-0.03	(-0.07 to 0.02)	0.211
Total body water	-0.03	(-0.08 to 0.03)	0.325
Extracellular water	-0.05	(-0.11 to 0.01)	0.113
Intracellular water	-0.01	(-0.07 to 0.04)	0.616

*Association had uncorrected P < 0.05
†Association had FDR-corrected P < 0.05



(C) Gray Matter Volume - Plasma Acetic Acid Levels

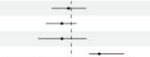
Variable	Beta	(95% CI)	Uncorrected *P*-value
Frontal lobe	0.03	(-0.04 to 0.10)	0.419
Temporal lobe	0.04	(-0.04 to 0.11)	0.329
Parietal lobe	0.06	(-0.02 to 0.14)	0.115
Occipital lobe	0.06	(-0.02 to 0.14)	0.114
Basal ganglia	0.02	(-0.08 to 0.13)	0.656
Thalamus	0.15	(0.05 to 0.26)	0.005 *,†
Cingulate	0.02	(-0.07 to 0.10)	0.691
Insula	0.04	(-0.05 to 0.14)	0.348

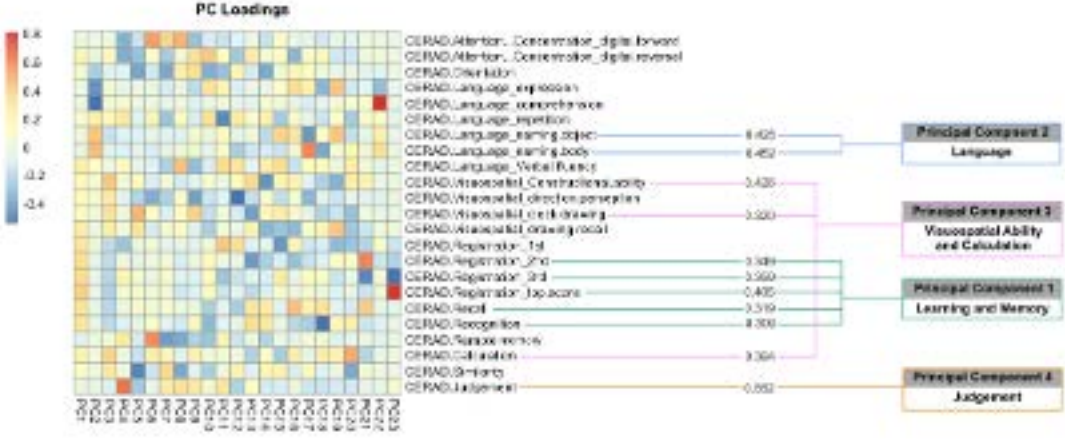
*Association had uncorrected P < 0.05
†Association had FDR-corrected P < 0.05

(D) Cognitive Function - Plasma Acetic Acid Levels

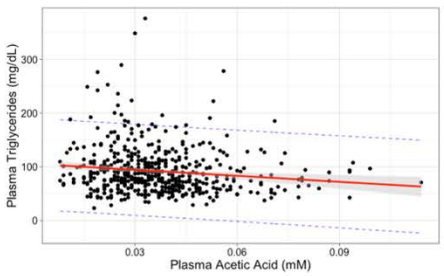
Variable	Beta	(95% CI)	Uncorrected *P*-value
PC1	-0.02	(-0.12 to 0.09)	0.778
PC2	-0.06	(-0.16 to 0.03)	0.138
PC3	-0.06	(-0.21 to 0.10)	0.426
PC4	0.18	(0.12 to 0.34)	0.001 *,†

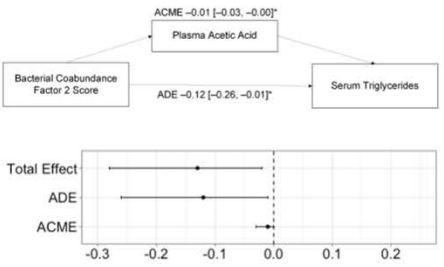
*Association had uncorrected P < 0.05
†Association had FDR-corrected P < 0.05



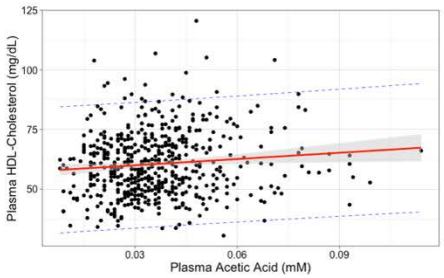


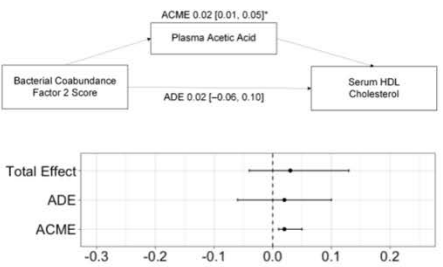
(A)


Plasma Triglycerides (mg/dL)
300
200
100
0
0.03
0.06
0.09
Plasma Acetic Acid (mM)

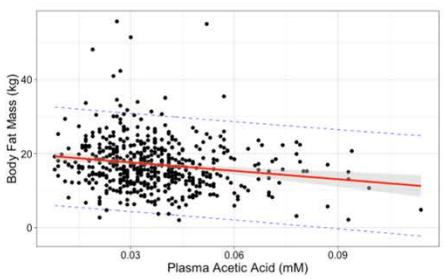

ACME −0.01 [−0.03, −0.00]*
Plasma Acetic Acid
Bacterial Coabundance Factor 2 Score
ADE −0.12 [−0.26, −0.01]*
Serum Triglycerides
Total Effect
ADE
ACME
-0.3
-0.2
-0.1
0.0
0.1
0.2

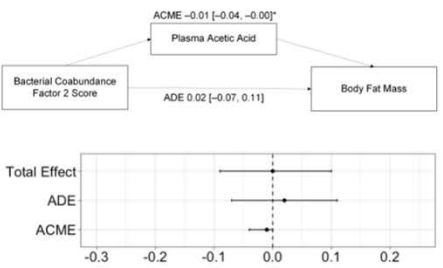
(B)


Plasma HDL-Cholesterol (mg/dL)
125
100
75
50
0.03
0.06
0.09
Plasma Acetic Acid (mM)

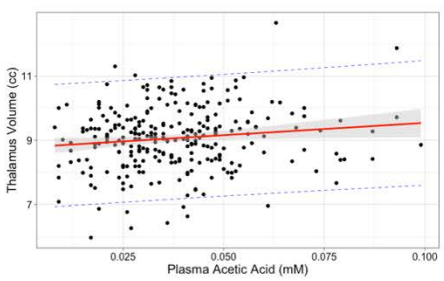

ACME 0.02 [0.01, 0.05]*
Plasma Acetic Acid
Bacterial Coabundance Factor 2 Score
ADE 0.02 [−0.06, 0.10]
Serum HDL Cholesterol
Total Effect
ADE
ACME
-0.3
-0.2
-0.1
0.0
0.1
0.2

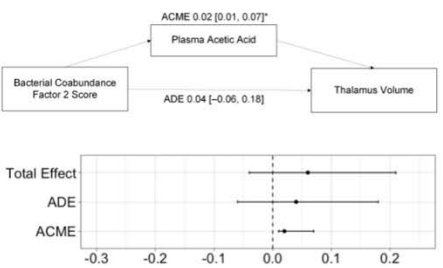
(C)


Body Fat Mass (kg)
40
20
0
0.03
0.06
0.09
Plasma Acetic Acid (mM)

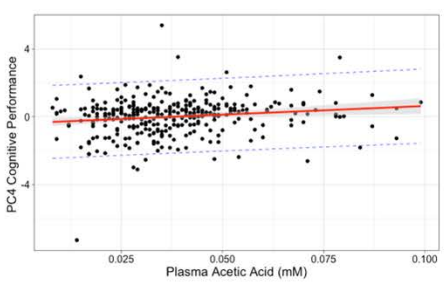

ACME −0.01 [−0.04, −0.00]*
Plasma Acetic Acid
Bacterial Coabundance Factor 2 Score
ADE 0.02 [−0.07, 0.11]
Body Fat Mass
Total Effect
ADE
ACME
-0.3
-0.2
-0.1
0.0
0.1
0.2

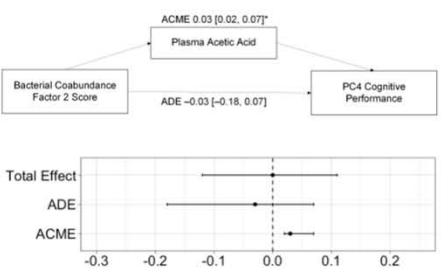
(D)


Thalamus Volume (cc)
11
9
7
0.025
0.050
0.075
0.100
Plasma Acetic Acid (mM)


ACME 0.02 [0.01, 0.07]*
Plasma Acetic Acid
Bacterial Coabundance Factor 2 Score
ADE 0.04 [−0.06, 0.18]
Thalamus Volume
Total Effect
ADE
ACME
-0.3
-0.2
-0.1
0.0
0.1
0.2

(E)


PC4 Cognitive Performance
4
0
-4
0.025
0.050
0.075
0.100
Plasma Acetic Acid (mM)


ACME 0.03 [0.02, 0.07]*
Plasma Acetic Acid
Bacterial Coabundance Factor 2 Score
ADE −0.03 [−0.18, 0.07]
PC4 Cognitive Performance
Total Effect
ADE
ACME
-0.3
-0.2
-0.1
0.0
0.1
0.2