Ni *et al. BMC Medicine* (2025) 23:669
https://doi.org/10.1186/s12916-025-04488-y

BMC Medicine

RESEARCH **Open Access**

Mediterranean diet, gut microbiota, and cognitive decline in older adults with obesity/overweight and metabolic syndrome: a prospective cohort study

Jiaqi Ni[1,2,3], Adrián Hernández-Cacho[1,2,3], Stephanie K. Nishi[1,2,3,4,5], Nancy Babio[1,2,3], Clara Belzer[6], Prokopis Konstati[6], Jesús Vioque[7,8], Dolores Corella[3,9], Olga Castañer[8,10], Josep Vidal[11,12], Isabel Moreno-Indias[3,13], Laura Torres-Collado[7,8], Oscar Coltell[3,14], Montse Fitó[3,10], Miguel Ruiz-Canela[3,15], Dong D. Wang[16,17,18], Francisco J. Tinahones[3,13] and Jordi Salas-Salvadó[1,2,3*]

Abstract

Background Emerging evidence highlights that diet dynamically shapes the gut microbiome, which in turn influences cognitive function through bidirectional gut-brain communication, offering a promising target for mitigating cognitive decline and neurodegenerative disorders. While the Mediterranean diet (MedDiet) is a well-established dietary pattern with demonstrated neuroprotective benefits, the interplay between MedDiet adherence, gut microbiota, and longitudinal cognitive trajectories remains poorly understood. We aimed to identify a gut microbial signature of the MedDiet adherence and prospectively examine the associations of MedDiet adherence and MedDiet gut microbial signature (MedDiet-GMS) with cognitive changes over time in older adults at high risk of cognitive decline.

Methods This study included 746 participants (mean age 65 ± 5 years, 48% women) with overweight/obesity and metabolic syndrome. Adherence to the MedDiet was assessed using a validated 14-item Mediterranean Diet Adherence Screener (MEDAS). Baseline gut microbiota composition was profiled via 16S rRNA sequencing. Cognitive function was evaluated at baseline, 2, 4, and 6 years using a comprehensive neuropsychological battery. Elastic net regressions were applied to derive a MedDiet-GMS, and linear mixed models were used to assess associations of both MEDAS and MedDiet-GMS with trajectories of cognitive function, adjusting for potential confounders.

Results Higher adherence to the MedDiet was associated with greater gut microbial diversity ($p < 0.05$) and distinct microbial composition (PERMANOVA, $p = 0.001$). The MedDiet-GMS comprised 20 taxa, including short-chain fatty acid-producers (e.g., *Barnesiella*, *Butyricicoccus*) positively weighted and pro-inflammatory taxa (e.g., *Eggerthella*) negatively weighted. Both higher MEDAS scores ($p = 0.007$) and MedDiet-GMS ($p = 0.036$) were independently associated with slower global cognitive decline. The MedDiet-GMS was additionally linked to preserved executive function ($p = 0.049$), while MEDAS was associated with attenuated general cognitive decline ($p = 0.028$). *Eggerthella*, inversely associated with MedDiet adherence, was linked to greater executive function decline (FDR < 0.05).

*Correspondence:
Jordi Salas-Salvadó
jordi.salas@urv.cat
Full list of author information is available at the end of the article

Conclusions Greater adherence to the MedDiet was associated with a favorable gut microbiota profile and slower cognitive decline over 6-year of follow-up. A microbiome-derived signature of MedDiet adherence was prospectively associated with favorable cognitive trajectories in older adults at risk of cognitive decline. External validation and experimental research are warranted to translate these findings into targeted microbiome-based dietary interventions for healthy cognitive aging.

Keywords Mediterranean diet (MedDiet), Gut microbiota, Cognitive decline, Cognitive function, Microbiota-gut-brain axis

Background

Cognitive functions, such as executive function, attention, language, learning and memory abilities, are essential for daily activities and are fundamental to maintaining independence and quality of life, particularly in older adults [1, 2]. While cognitive decline is a natural process of aging, accelerated deterioration characterizes neurodegenerative disorders such as mild cognitive impairment (MCI) and dementia [1]. With the rapid increase in the aging population, these cognitive-deficit conditions represent a growing global public health concern and economic burden [3]. Therefore, identifying cost-effective strategies to delay or prevent the onset of MCI and dementia or mitigate cognitive decline has become a major public health priority [1, 3]. Numerous modifiable risk factors associated with dementia have been identified [1], many of which are inter-related and are closely linked to dietary habits, making diet a particularly promising target due to its accessibility and potential to deliver a cascade of health benefits for aging populations at risk of dementia [1, 3, 4].

The Mediterranean diet (MedDiet), characterized by a high consumption of plant-based foods, e.g., vegetables, fruits, nuts, legumes, and whole grains, the use of extra virgin olive oil as the main fat source, and moderate consumption of fish and wine, with limited intake of red and processed meat, dairy products, sweetened beverages, and saturated fats, has been associated with better cognitive outcomes, including a reduced risk of cognitive impairment and dementia, as well as a slower rate of cognitive decline, in both observational studies and randomized controlled trials (RCTs) [5, 6]. The mechanisms underlying the neuroprotective effects of the MedDiet still remain unclear, but growing evidence points to the gut-brain axis as a key pathway [7]. The gut microbiota, a complex and dynamic community of microorganisms inhabiting the gastrointestinal tract, plays a crucial role in regulating brain function through immune, neuroendocrine, vagus nerve, and metabolic pathways [8]. Diet is one of the most influential modulators of the gut microbiota, capable of inducing substantial changes in microbial diversity and the abundance of specific taxa [9–11]. A previous RCT demonstrated that adherence to a modified MedDiet can alter gut microbiota composition that correlated with cerebrospinal fluid biomarkers of Alzheimer's disease such as amyloid-β (Aβ42) in adults with MCI [12]. Furthermore, in pre-frail older adults, 12-month adherence to the MedDiet was associated with improvements in cognitive performance, which were linked to diet-induced enrichment of butyrogenic taxa, microbes known to produce bioactive compounds with potential neuroprotective properties [13].

Despite the emerging interest in microbiota-targeted nutritional strategies for cognitive health [7, 14], few studies have concurrently examined dietary patterns, gut microbiota composition, and cognitive trajectories in aging populations [12, 15]. Studying populations at elevated risk of cognitive decline may provide important insights, as preventive strategies are most relevant in those most vulnerable. Therefore, in the present study, we investigated the associations between MedDiet, gut microbiota composition, and changes in cognitive performance over a 6-year of follow-up in cognitively healthy older adults with overweight or obesity and metabolic syndrome (MetS) at baseline, conditions that are well-established modifiable risk factors for dementia [1, 3, 16, 17]. Thus, our cohort provides a unique opportunity to evaluate whether MedDiet and its microbiome correlates may confer protective benefits in a relatively high-risk context. Specifically, our aims were to as follows: (1) examine the cross-sectional association between MedDiet adherence and gut microbiota composition, and to derive a gut microbiota signature score correlated with adherence to the MedDiet (MedDiet-GMS); and (2) prospectively assess the associations of both self-reported MedDiet adherence and the derived MedDiet-GMS with cognitive changes over time. We hypothesized that higher adherence to the MedDiet would be associated with gut microbiota characteristics supportive of host health, and that both dietary adherence and the gut microbial signature of MedDiet would be associated with a slower rate of cognitive decline.

Methods

Study design and participants

PREDIMED-Plus (PREvención con DIeta MEDiterránea-Plus) is a 6-year, multicenter, randomized controlled lifestyle trial for prevention of a composite cardiovascular endpoint. Neurodegenerative diseases and changes in cognitive function are pre-defined secondary and intermediate outcomes in this trial. This trial enrolled men and women aged 55–75 years with overweight or obesity and MetS [18]. Briefly, exclusion criteria included previous history of CVD, any chronic medical condition, psychiatric disorders, dementia, institutionalization, and any allergy to MedDiet-related foods. Eligible participants were randomized in a 1:1 ratio to either an energy-reduced Mediterranean diet (erMedDiet) with physical activity promotion and behavioral support (intervention group) or a control group following ad libitum MedDiet recommendations with usual care. All participants provided written informed consent, and the trial protocol was approved by the research ethics committees of all participating institutions. Details of the study design are available online (https://www.predimedplus.com/) and in previous publications [19–21]. The trial was registered at the International Standard Randomized Controlled Trial (Number: ISRCTN89898870) in 2014.

The present study included 746 participants who had baseline stool samples for microbial profiling, collected at least 15 days after the cessation of antibiotic treatment when applicable. All these participants also completed dietary and cognitive function assessments at baseline and at least one follow-up visit. A detailed overview of the present study population selection and design is provided in Additional file 1: Fig. S1 and Fig. 1, respectively.

Cognitive function assessment

Cognitive function was evaluated at baseline and during follow-up visits at years 2, 4, and 6 using a comprehensive battery of eight neuropsychological tests. These assessments included the Mini-Mental State Examination (MMSE) [22, 23], Clock Drawing Test (CDT) [24–26], Verbal Fluency Tests (VFT) for Animals and Letter "P" [27, 28], Digit Span Test (DST)-Forward and DST-Backward from the Wechsler Adult Intelligence Scale-III (WAIS-III) [29–31], and Trail Making Tests (TMT) parts A and B [31–33]. A detailed description of these neuropsychological tests and the methodology used to generate cohort-specific z-scores has been previously published [34].

To assess overall cognitive performance, a global cognitive function (GCF) score was derived by averaging all individual test z-scores, adding or subtracting based on whether higher values indicate better or worse cognitive performance. Since MMSE and CDT serve as multidomain screening tools, an additional general cognitive function composite score was generated as a screening-summary measure. Furthermore, three domain-specific composite scores, i.e., executive function, attention, and language, were calculated by averaging the z-scores of relevant tests. A comprehensive explanation of the composite score calculations can be found in Additional file 1: TableS1. The GCF and general cognitive function scores, as well as domain-specific cognitive composite scores at each visit were standardized to z-scores using the mean and standard deviation of the respective baseline composite score, where higher values represent better cognitive performance.

Dietary assessment

Habitual dietary intake was evaluated at baseline and annually during follow-up using a validated, semi-quantitative Food Frequency Questionnaire (FFQ) [35]. This questionnaire included predefined portion sizes for each food item, with nine frequency options ranging from "never or almost never" to "≥ 6 times/day." Total energy and nutrients intake were estimated from Spanish food composition tables [36, 37]. Adherence to the traditional MedDiet was assessed using a validated 14-item Mediterranean Diet Adherence Screener (MEDAS) [38]. The screener consists of 14 dichotomous questions on habitual intake of several food items (Additional file 1: TableS2): habitual consumption of olive oil (2 questions), fruits, vegetables, red/processed meat, butter/margarine/cream, sugar-sweetened beverages (SSB), wine, legumes, seafood, sweets/pastries, nuts, whether participants prefer white meat over red meat, and consumption of sofrito as seasoned sauce. A score of 1 was assigned for each criterion met, with a total MEDAS score ranging from 0 to 14, where higher values indicate greater adherence to the MedDiet. In the control group, the 14-item MEDAS was directly administered by trained dietitians during in-person visits at baseline and annually. In the intervention group, adherence was assessed using a validated 17-item energy-restricted MEDAS (er-MEDAS) instead [39]. The er-MEDAS is a modification of the original 14-item MEDAS designed to capture moderation of food intake to reflect energy restriction. Most items from the original MEDAS were retained with slight modifications, while four new items were added and one was removed (Additional file 1: TableS3). In our dataset, among participants with both assessments available, the correlation between er-MEDAS and MEDAS scores was high ($r = 0.71$, $p < 0.001$), supporting their comparability. Therefore, for harmonization, an equivalent 14-point MEDAS score was derived post hoc in the intervention group using corresponding items from the er-MEDAS and FFQ [40]. To align dietary assessments with cognitive function measurements and minimize potential bias from the intervention over time, MEDAS scores from baseline, year 2, year 4, and year 6 visits were used in this analysis.

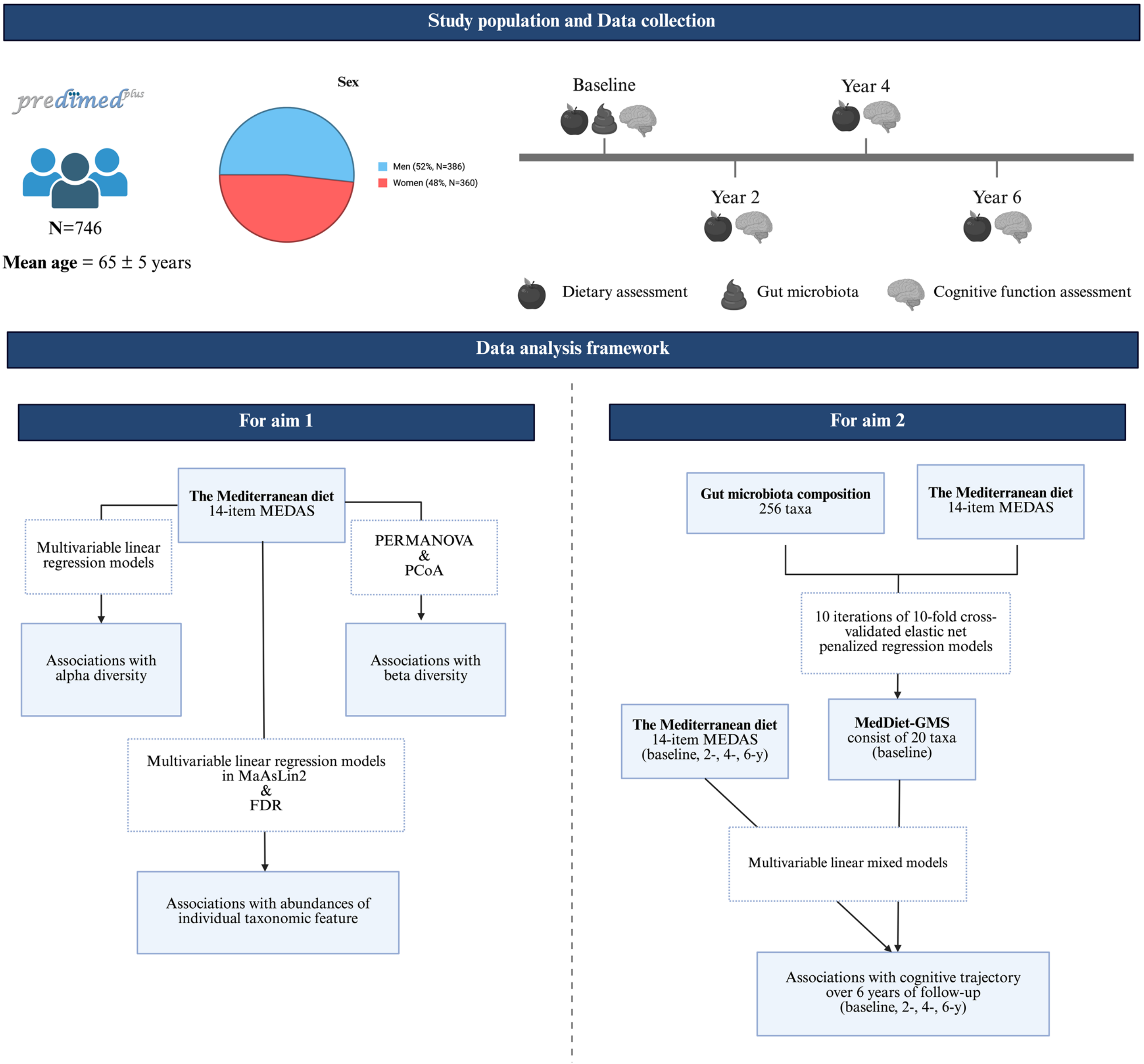

Fig. 1 Study population, data collection and analysis strategy. Abbreviations: FDR, false discovery rate; MaAsLin2, Microbiome Multivariable Association with Linear Models; MEDAS, Mediterranean Diet Adherence Screener; MedDiet, Mediterranean diet; MedDiet-GMS, Mediterranean diet related gut microbial score; PCoA, principal coordinate analysis; PERMANOVA, permutational multivariate analysis of variance

DNA extraction and microbiome profiling

The methods for stool sample collection, microbial DNA extraction, 16S ribosomal RNA (rRNA) amplicon sequencing, and bioinformatic processing have been previously detailed [41]. In summary, participants collected stool samples at home following standardized protocols accompanied by illustrated instructions at baseline. Stool samples were aliquoted and stored at − 80 °C until microbial DNA extraction. The extracted DNA was assessed for quality and concentration before sequencing. The V4 region of the 16S rRNA gene was amplified using triplicate polymerase chain reaction (PCR), and the resulting amplicons were sequenced on the Illumina NovaSeq platform. Quality control procedures included the incorporation of artificial mock communities with known composition as positive controls and the use of negative control samples to identify potential contaminant sequences. Raw sequencing data were processed using the DADA2 pipeline under default settings [42], which involved demultiplexing paired-end reads, quality filtering, and the generation of amplicon sequence variants (ASVs). Taxonomic assignment of ASVs was performed using the Silva reference database (v138.1) [43]. A total of 1.080.337 paired-end reads per sample on average (median, 1.120.481; IQR, [379.416–1.645.145]) were generated across all study samples. After filtering, denoising, and chimera removal, an average of 948.831 non-chimeric reads per sample (median, 969.828; IQR, [334.276–1.415.867]) were retained, with 11% of sequences identified as chimeric and removed. Importantly, no samples were excluded due to low sequencing depth, as all exceeded a 10.000-read threshold that was used in this study.

Assessment of covariates

Sociodemographic information including age, sex, education level, and marital status, as well as lifestyle factors such as smoking status and physical activity were collected by trained personnel via interviewer-administered questionnaires. Physical activity was assessed using a validated Spanish short version of the Minnesota Leisure Time Physical Activity Questionnaire (the REGICOR questionnaire) [44]. Medical history (e.g., prevalence of type 2 diabetes, hypertension, and hypercholesterolemia), along with medication use, was obtained from participant self-reports or collected from medical records. Depressive symptomatology was evaluated using the Beck Depression Inventory (BDI-II), with a score of ≥ 14 indicating a risk of depression [45, 46]. Anthropometric measurements were performed by trained personnel following standardized procedures. Weight and height were recorded using calibrated scales and wall-mounted stadiometers, with participants wearing light clothing and no footwear or accessories. Body mass index (BMI) was calculated as weight in kilograms divided by height in meters squared. Waist circumference was measured at the midpoint between the lowest rib and the iliac crest using a flexible anthropometric tape.

Statistical analysis

Baseline characteristics of the study population

Baseline characteristics of the study population were described according to lower versus higher adherence to the MedDiet, as assessed by the MEDAS and/or MedDiet-GMS scores, and presented using descriptive statistics (i.e., means, medians, and proportions).

Associations between adherence to the MedDiet and gut microbiota at baseline

Multivariable linear regression models were used to examine the association between MedDiet adherence assessed by MEDAS and gut microbial alpha diversity indices (Chao1, Inverse Simpson, Shannon) [47–49], adjusting for age, sex, recruiting center, BMI, physical activity, smoking status, total energy intake, depressive symptomatology, prevalence of diabetes, hypertension, and hypercholesterolemia (Additional file 1: Fig. S2). Alpha diversity indices were standardized to z-scores to facilitate comparability. To determine variability in overall gut microbial structure, we calculated the Aitchison distance metric for each sample, a Euclidean distance metric based on centered log-ratio (clr) transformed abundance counts [50]. Principal coordinate analysis (PCoA) was conducted based on the Aitchison distance to detect potential intrinsic patterns of microbial community structure, with the first two PCoA axes (PCoA1 and PCoA2) extracted to summarize the primary variation in microbial community composition. Permutational multivariate analysis of variance (PERMANOVA; $n = 999$ permutations) [51] using "adonis2" function of vegan package was applied to quantify the percentage of variance in the microbial taxonomy explained by dietary variables, baseline cognitive function, and covariables based on Aitchison dissimilarity metric. Additionally, PERMANOVA assessed cross-sectional differences in microbiota dissimilarity at baseline MEDAS levels, adjusting for the same covariates used in the alpha diversity analysis. For per-feature analysis, microbial features at the genus level detected at a relative abundance of at least 0.1% in at least 10% of all samples were included in the analysis. Multivariable linear regression models implemented in MaAsLin2 [52] were used to identify microbial taxa associated with MEDAS. Default MaAsLin2 settings were applied, models were adjusted for the same covariates as mentioned above. p-values were adjusted using the Benjamini–Hochberg method. In line with previous microbiome studies [53, 54], we reported results at an FDR threshold of 0.25 to identify suggestive associations, used for exploratory purposes to enable comparability with prior work and to highlight potential signals warranting further validation, while statistical significance in our study was defined as FDR (q value) < 0.05.

Construction of Mediterranean diet related gut microbial signature score (MedDiet-GMS)

We applied elastic net penalized regression models as a dimension-reduction approach, regressing gut microbial genera on the MEDAS score at baseline to construct a Mediterranean diet related gut microbial signature score (MedDiet-GMS). The relative abundances of filtered microbial genera were transformed using total sum scaling, log-transformed, and subsequently standardized (z-scored) to ensure all features were on the same scale before including them in the models. This preprocessing approach mirrors the filtering, transformation and scaling procedures applied in MaAsLin2 for per-feature microbiome analysis, ensuring consistency in data treatment. We performed 10 iterations of tenfold cross-validated elastic net regression. In each iteration, the optimal combination of alpha and lambda parameters was selected via internal cross-validation on

90% of the data. The final model for each iteration was refitted using these optimal parameters, and microbial genera with non-zero coefficients were extracted. We retained only genera that were consistently selected (non-zero coefficient) in all 10 iterations. Their coefficients were then averaged and used to construct the final MedDiet-GMS. The association between each individual genus constituting the MedDiet-GMS and the MEDAS score was further assessed using multivariable linear regression. The Benjamini–Hochberg method was used to correct for multiple testing, with a false discovery rate (FDR) threshold of < 0.05 considered statistically significant.

Associations between adherence to the MedDiet and trajectories of cognitive function over time

Multivariable linear mixed-effects models (LMMs) were used to evaluate the association between MedDiet adherence and trajectories of cognitive function over 6 years of follow-up. Adherence to the MedDiet was assessed using repeated MEDAS scores at each follow-up visit, so the MEDAS exposure was updated over time, and/or baseline microbiome predicted MedDiet adherence score (MedDiet-GMS). Given the nonlinear trajectory of cognitive function over time, follow-up visit was modeled as a categorical variable. Cognitive function at baseline was modeled as visit = 0, with random intercept specified at the participant level to account for inter-individual variability in baseline performance. Three models were constructed: model 1: included fixed effects for MEDAS or MedDiet-GMS, visit, and their interaction term (MEDAS*visit or MedDiet-GMS*visit); model 2: further adjusted for age (years), sex (male or female), recruiting center (Alicante, Barcelona, Reus, or Valencia), intervention group; model 3: additionally adjusted for educational level (primary, secondary, or college), marital status (single, divorced, separated, married, or widowed), as well as baseline BMI (kg/m^2), physical activity (MET-min/day), smoking status (never, former, or current smoker), total energy intake (kcal/day), depressive symptomatology (yes or no), along with baseline prevalence of diabetes (yes or no), hypertension (yes or no), and hypercholesterolemia (yes or no) (Additional file 1: Fig. S2). The general specification of the Models can be written as follows:

$$\begin{aligned} \mathit{Cognitive\ function\_ij} &= \beta_0 + \beta_1 \times \mathit{MEDAS\_ij} + \beta_2 \times \mathit{Visit\_ij} \\ &\quad + \beta_3 \times (\mathit{MEDAS\_ij} \times \mathit{Visit\_ij}) + u\_i + \varepsilon\_ij, \end{aligned}$$

$$\begin{aligned} \mathit{Cognitive\ function\_ij} &= \beta_0 + \beta_1 \times \mathit{MedDiet} - \mathit{GMS\_i} + \beta_2 \times \mathit{Visit\_ij} \\ &\quad + \beta_3 \times (\mathit{MedDiet} - \mathit{GMS\_i} \times \mathit{Visit\_ij}) + u\_i + \varepsilon\_ij \end{aligned}$$

where:

- i: participants
- j: visit/timepoint (baseline, 2 years, 4 years, 6 years)
- β_0: overall intercept (mean cognitive function at baseline)
- β_3: interaction effect (how the association between MEDAS or MedDiet-GMS modifies cognitive trajectories over time
- u_i: participant-specific random intercept
- ε_ij: residual error

For the MEDAS models, the exposure variable was updated at each visit (time-varying), whereas MedDiet-GMS was estimated at baseline only.

Cognitive decline was operationalized as the trajectory of repeated cognitive assessments and the interaction term between exposure and visit (β_3) represents how MedDiet adherence was associated with the rate of cognitive change over time.

In addition, associations between individual genera constituting the MedDiet-GMS and trajectory of cognitive function were also examined using LMMs with the same adjustments. Multiple testing correction was applied using the Benjamini–Hochberg procedure, and associations with FDR < 0.05 were considered statistically significant.

Missing data in baseline covariates were minimal, with one missing value each for civil and smoking status, which were imputed using the most frequent category. No imputations were performed for cognitive function outcomes. All statistical analyses were performed using R (version 4.3.1). A two-tailed p-value or FDR (q value) < 0.05 was considered statistically significant.

Results

Study population characteristics

Figure 1 provides an overview of the present study, while the inclusion flowchart of the study population is illustrated in Additional file 1: Fig. S1. A total of 746 participants were included in the analysis, with a mean age of 65 ± 5 years, of whom 48% were women. Baseline characteristics of the study population were described for the entire cohort analyzed and stratified into 2 groups based on adherence to the MedDiet, assessed either by the MEDAS or predicted by the MedDiet-GMS (Table 1 and Additional file 1: TableS4). The mean MEDAS score for the total study population was 8.42 ± 1.78, with the median (range) MEDAS scores being 7 (2–8) and 10 (8–13) for the lower and higher adherence groups, respectively. When predicted by the MedDiet-GMS, the lower and higher adherence group scores were 8.1 (4.4–8.4) and 8.8 (8.4–11.6),

Table 1 Baseline characteristics of the study population

	Total	**MEDAS**		**MedDiet-GMS**	
		Lower **6.98 (1.08)** **Median (range): 7 (2–8)**	**Higher** **9.87 (1.01)** **Median (range): 10 (8–13)**	**Lower** **7.95 (0.52)** **Median (range): 8.06 (4.44–8.42)**	**Higher** **8.90 (0.43)** **Median (range): 8.80 (8.42–11.60)**
No. of participants	746	373	373	373	373
Socio-demographic variables					
Age, years	64.89 (4.92)	64.21 (5.18)	65.56 (4.55)	64.43 (5.10)	65.35 (4.70)
Women, *n* (%)	360 (48.3)	167 (44.8)	193 (51.7)	161 (43.2)	199 (53.4)
Education level, *n* (%)					
Primary or less	396 (53.1)	191 (51.2)	205 (55.0)	201 (53.9)	195 (52.3)
Secondary	212 (28.4)	111 (29.8)	101 (27.1)	104 (27.9)	108 (29.0)
College	138 (18.5)	71 (19.0)	67 (18.0)	68 (18.2)	70 (18.8)
Marital status, *n* (%)					
Single, divorced or separated	99 (13.3)	49 (13.1)	50 (13.4)	52 (13.9)	47 (12.6)
Married	575 (77.1)	289 (77.5)	286 (76.7)	282 (75.6)	293 (78.6)
Widower	72 (9.7)	35 (9.4)	37 (9.9)	39 (10.5)	33 (8.8)
Disease present at recruitment					
Type 2 diabetes, *n* (%)	204 (27.3)	102 (27.3)	102 (27.3)	115 (30.8)	89 (23.9)
Hypertension, *n* (%)	606 (81.2)	302 (81.0)	304 (81.5)	305 (81.8)	301 (80.7)
Hypercholesterolemia, *n* (%)	493 (66.1)	247 (66.2)	246 (66.0)	247 (66.2)	246 (66.0)
Depressive symptomatology, *n* (%)	145 (19.4)	77 (20.6)	68 (18.2)	84 (22.5)	61 (16.4)
Medication use, *n* (%)					
Insulin or other antidiabetic drugs	172 (23.1)	86 (23.1)	86 (23.1)	99 (26.5)	73 (19.6)
Antihypertensive agents	578 (77.5)	283 (75.9)	295 (79.1)	292 (78.3)	286 (76.7)
Statins or other hypolipidemic drugs	364 (48.8)	182 (48.8)	182 (48.8)	183 (49.1)	181 (48.5)
Antidepressant	166 (22.3)	90 (24.1)	76 (20.4)	89 (23.9)	77 (20.6)
Anthropometric variables					
BMI, kg/m^2	32.83 (3.51)	33.12 (3.51)	32.53 (3.48)	32.97 (3.46)	32.68 (3.55)
Waist circumference, cm					
Women	104 (10)	105 (10)	103 (10)	104 (9)	104 (10)
Men	111 (9)	111 (9)	111 (9)	112 (9)	110 (9)
Lifestyle variables					
Smoking status, *n* (%)					
Never smoked	358 (48.0)	173 (46.4)	185 (49.6)	162 (43.4)	196 (52.5)
Former smoker	292 (39.1)	145 (38.9)	147 (39.4)	158 (42.4)	134 (35.9)
Current smoker	96 (12.9)	55 (14.7)	41 (11.0)	53 (14.2)	43 (11.5)
Physical activity, METs/min/day	360.85 (344.04)	331.08 (324.78)	390.61 (360.27)	320.87 (329.66)	400.82 (353.79)
Total alcohol intake, g/day	10.81 (13.78)	10.54 (14.51)	11.09 (13.02)	10.95 (13.81)	10.67 (13.77)
Cognitive performance assessment, raw scores					
Mini-Mental State Examination	28.57 (1.63)	28.47 (1.79)	28.67 (1.45)	28.55 (1.62)	28.59 (1.64)
Clock Drawing Test	5.90 (1.23)	5.85 (1.29)	5.95 (1.17)	5.88 (1.27)	5.92 (1.20)
Verbal fluency tasks semantical	15.67 (4.52)	15.73 (4.69)	15.61 (4.35)	15.85 (4.67)	15.49 (4.36)
Verbal fluency tasks phonological	11.98 (4.13)	11.99 (4.29)	11.96 (3.98)	11.89 (4.29)	12.06 (3.97)
Trail making test part A[a]	53.47 (30.41)	55.92 (36.50)	51.01 (22.53)	54.58 (34.70)	52.36 (25.44)
Trail making test part B[a]	128.97 (65.99)	131.26 (70.87)	126.67 (60.72)	129.54 (69.02)	128.40 (62.91)
Digit Span Test forward	9.06 (2.55)	9.14 (2.49)	8.98 (2.62)	9.25 (2.58)	8.88 (2.52)
Digit Span Test backward	5.46 (2.28)	5.51 (2.27)	5.40 (2.29)	5.66 (2.29)	5.25 (2.26)

Table 1 (continued)

	Total	MEDAS		MedDiet-GMS	
		Lower **6.98 (1.08)** **Median (range): 7 (2–8)**	**Higher** **9.87 (1.01)** **Median (range): 10 (8–13)**	**Lower** **7.95 (0.52)** **Median (range): 8.06 (4.44–8.42)**	**Higher** **8.90 (0.43)** **Median (range): 8.80 (8.42–11.60)**
Dietary consumption					
Total energy intake, kcal/day	2464.38 (497.47)	2464.60 (533.10)	2464.16 (459.82)	2460.43 (504.62)	2468.33 (490.87)
Fiber, g/day	26.80 (8.18)	25.09 (7.63)	28.51 (8.36)	25.58 (7.70)	28.01 (8.46)
Vegetables, g/day	345.91 (140.21)	330.33 (139.68)	361.48 (139.20)	335.89 (139.47)	355.92 (140.42)
Fruits, g/day	358.51 (199.06)	332.33 (187.27)	384.70 (207.15)	341.58 (190.57)	375.44 (206.06)
Legumes, g/day	19.77 (10.36)	18.26 (9.47)	21.28 (10.98)	19.04 (9.44)	20.51 (11.16)
Total cereals, g/day	159.14 (70.25)	161.83 (73.91)	156.44 (66.37)	162.17 (71.26)	156.10 (69.18)
Whole grains, g/day	40.22 (62.33)	34.75 (57.75)	45.68 (66.22)	34.38 (59.60)	46.05 (64.49)
Total meat, g/day	159.87 (55.66)	166.75 (57.44)	152.99 (53.01)	162.76 (56.53)	156.99 (54.70)
Red/processed meat, g/day	105.06 (48.18)	112.14 (51.13)	97.98 (43.97)	108.07 (49.00)	102.05 (47.22)
Total fish, g/day	107.74 (46.12)	99.04 (41.43)	116.44 (48.91)	103.24 (45.68)	112.24 (46.19)
Total dairy, g/day	307.14 (177.70)	315.75 (179.95)	298.52 (175.25)	320.47 (185.81)	293.80 (168.40)
Confectionery products, g/day	24.53 (26.39)	29.27 (29.07)	19.78 (22.46)	26.97 (28.39)	22.09 (24.02)
Total olive oil, g/day	45.57 (14.55)	41.63 (14.65)	49.50 (13.36)	43.46 (14.52)	47.68 (14.29)
Butter/margarine, g/day	1.13 (2.95)	1.58 (3.82)	0.68 (1.56)	1.32 (3.29)	0.94 (2.56)
Total nuts, g/day	16.57 (15.94)	13.23 (14.67)	19.91 (16.46)	14.67 (14.74)	18.46 (16.86)
Coffee, mL/day	41.74 (52.77)	41.92 (52.77)	41.55 (52.84)	39.35 (50.91)	44.12 (54.53)
Tea, mL/day	9.56 (26.07)	10.20 (30.47)	8.93 (20.78)	9.29 (24.66)	9.84 (27.43)
SSB, mL/day	37.84 (78.19)	48.25 (94.15)	27.43 (56.24)	43.98 (92.79)	31.70 (59.66)
Wine, mL/day	59.49 (86.92)	50.78 (85.73)	68.19 (87.35)	57.59 (85.61)	61.39 (88.28)

Data are presented as n (%) or mean (SD) for categorical or continuous variables, respectively

p-value for differences between groups was calculated by Pearson's chi-square test or Student's *t* test, as appropriate

BMI body mass index, *IQR* interquartile range, *MEDAS* Mediterranean Diet Adherence Screener, *MedDiet-GMS* Mediterranean diet related gut microbial score, *METs* metabolic equivalents, *SSB* sugar sweetened beverage

[a] Inverse neuropsychological assessment score, lower scores represent better cognitive performance

respectively. Baseline characteristics were generally comparable between the two classification methods. Participants with higher adherence to the MedDiet, whether assessed by a higher MEDAS score or a higher MedDiet-GMS score, tended to be older, more physically active, and had higher consumption of key beneficial MedDiet components, including fruits, whole grains, fish, total olive oil, and nuts, along with lower consumption of red/processed meat, sweets and sugar sweetened beverages (Table 1 and Additional file 1: TableS4).

Gut microbiota taxonomy profile of the Mediterranean diet

Higher adherence to the MedDiet was associated with greater α-diversity, as indicated by higher inverse Simpson and Shannon indices in the fully adjusted models, reflecting a greater richness and diversity of the gut microbiota composition (Fig. 2a). PERMANOVA revealed that MEDAS was significantly associated with diversity of the gut microbiota composition (p-value = 0.001; Fig. 2b), although it was not a major driver of overall variation, explaining 0.3% of variance (Fig. 2c). To identify microbial features associated with MedDiet and its individual components, we performed per-feature testing in MaAsLin2 using multivariable linear regression models. A total of 113 microbial features were associated with either MEDAS total score or one of its 14 individual components, with 29 taxa associated with the MEDAS total score only ($q < 0.25$; Fig. 2d). In general, we observed that the associations of taxa abundance with plant-based foods were in the opposite direction compared to those with red meat. Among the components of the MEDAS, olive oil, vegetables, red meat, and wine were the major contributors to the associations between the overall dietary pattern and the taxonomic features (Fig. 2d).

We applied an elastic net model as a dimension-reduction approach, regressing the taxa on the MEDAS

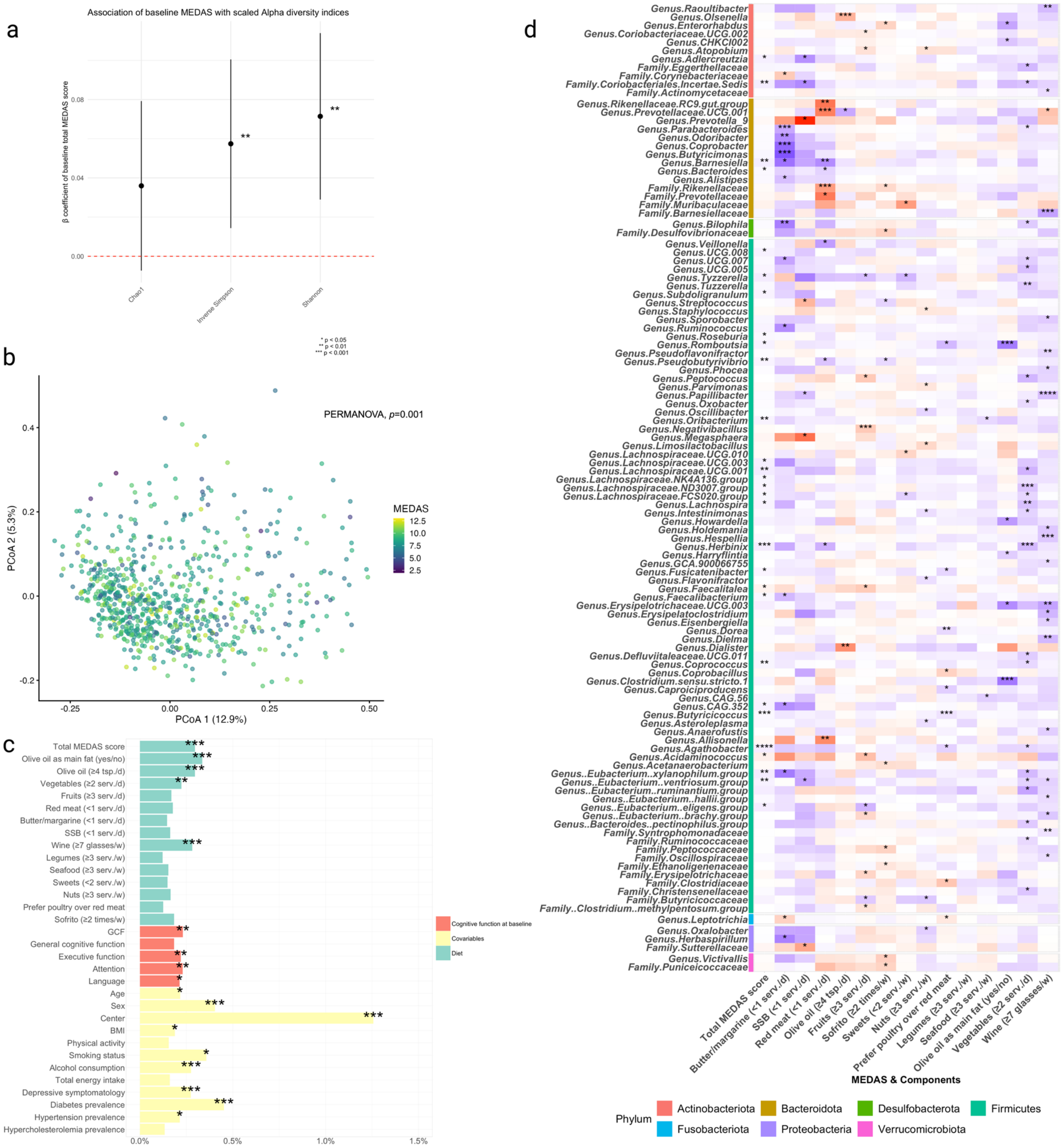

Fig. 2 Mediterranean diet adherence and gut microbiota composition. **a** Associations between MEDAS and alpha diversity metrics assessed using linear regression models adjusted for age (years); sex (male or female); recruiting center (Alicante, Barcelona, Reus, or Valencia); BMI (kg/m^2); physical activity (METs-min/day); smoking status (never, former, or current smoker); total energy intake (kcal/day); depressive symptomatology (yes or no); prevalence of diabetes (yes or no); hypertension (yes or no); and hypercholesterolemia (yes or no). **b** Principal coordinate analysis of all samples using ASV-level Aitchison distance dissimilarity. Results of PERMANOVA analysis assessing cross-sectional differences in microbiota dissimilarity at baseline MEDAS levels are provided. **c** Proportion of variation in taxonomy explained by the MEDAS and its components, cognitive function at baseline and covariables as quantified by two-sided permutational multivariate analysis of variance (based on ASV-level Aitchison distance dissimilarity). The *q* values (false discovery rate adjusted *p*-value) were calculated using the Benjamini–Hochberg method, ***q value < 0.005, **q value < 0.01, *q value < 0.05. **d** Associations of the MEDAS and its components with genus-level features. Colors of the heatmap are in correspondence to the beta coefficient for dietary variables from linear regression models in MaAsLin2 with genus-level feature as outcomes. All models were adjusted for the same covariables as previously mentioned. Statistical significance is from the linear regression model with multiple comparison adjustment using the Benjamini–Hochberg method to calculate *q* value, ****q value < 0.01, ***$0.01 \leq q$ value < 0.05, **$0.05 \leq q$ value < 0.1, *$0.1 \leq q$ value < 0.25. Abbreviations: GCF, global cognitive function; MEDAS, Mediterranean Diet Adherence Screener; PCoA, principal coordinate analysis; PERMANOVA, permutational multivariate analysis of variance; SSB, sugar sweetened beverages

to construct a gut microbial signature score (MedDiet-GMS) for the Mediterranean diet. Model performance was modest, with $R^2 = 0.15$ and cross-validated $Q^2 = 0.03$, though with limited predictive ability but appropriate for identifying robust taxa associated with MedDiet adherence. Among the 256 taxa, 20 were selected for the MedDiet-GMS construction, with positive weights assigned to genera such as *Barnesiella*, *Butyricicoccus*, and *Bacteroides*, while negative weights assigned to genera including *Latilactobacullus* and *Bifidobacterium* (Additional file 1: Fig. S3). The MedDiet-GMS was significantly correlated with the MEDAS (Pearson's $r = 0.38$, p-value < 0.001; Additional file 1: Fig. S4). As expected, and similar to observations with MEDAS, a higher MedDiet-GMS was associated with greater α-diversity across all three indices (Chao1, Inverse Simpson, Shannon), indicating higher richness and diversity (Additional file 1: Fig. S5a). Moreover, PERMANOVA revealed a significant association between MedDiet-GMS and overall structural variation of the gut microbiota composition (p-value $= 0.001$) (Additional file 1: Fig. S5b). In per-feature testing in MaAsLin2 using multivariable linear regression models, 133 taxa were associated with MedDiet-GMS, and generally following the same directional patterns observed with MEDAS ($q < 0.25$; Additional file 1: Fig. S6a). Considering all three sets together (MEDAS total score, MEDAS components, MedDiet-GMS), 171 taxa were identified ($q < 0.25$; Additional file 1: Fig. S6a). At the stricter threshold of $q < 0.05$, 3 taxa were significantly associated with MEDAS total score only, and 91 taxa with the MedDiet-GMS only. The overlap of taxa associated with MEDAS and MedDiet-GMS is illustrated at thresholds $q < 0.25$ (Additional file 1: Fig. S6b) and $q < 0.05$ (Additional file 1: Fig. S6c).

Associations with cognitive trajectories over time

We assessed the associations of the MEDAS and MedDiet-GMS with trajectories of cognitive function z-scores over 6 years using multivariable LMMs. Results from the fully adjusted models (Model 3) are presented in the Fig. 3, with estimates from all models shown in Additional file1: TableS5. Higher scores of both MEDAS and MedDiet-GMS were associated with a more favorable trajectory in global cognitive function over time (MEDAS*visit, overall p-value $= 0.0074$; MedDiet-GMS*visit, overall p-value $= 0.0355$ respectively in the fully adjusted models). Specifically, per 1-point higher MEDAS was associated with 0.05 (95% CI, 0.02–0.08) higher standardized global cognitive scores at year 2 compared to baseline and 0.04 (95% CI, 0.01–0.07) at year 4. Similarly, each 1-point higher MedDiet-GMS was associated with 0.1 (95% CI, 0.03–0.17) higher standardized global cognitive scores at year 4 versus baseline and 0.08 (95% CI, 0.01–0.16) at year 6 (Additional file 1: TableS5, Model 3). Domain-specific analyses further showed that in the fully adjusted models, higher MEDAS score was significantly associated with attenuated decline in general cognitive function (Year 2 vs Baseline: $\beta = 0.06$, 95% CI $= 0.01–0.11$; Year 4 vs Baseline: $\beta = 0.08$, 95% CI $= 0.02–0.13$; overall p-value $= 0.0278$), whereas higher MedDiet-GMS score was significantly associated with slower decline in executive function (Year 4 vs Baseline: $\beta = 0.09$, 95% CI $= 0.02–0.16$; Year 6 vs Baseline: $\beta = 0.08$, 95% CI $= 0.01–0.16$; overall p-value $= 0.0487$) over 6 years of follow-up. No significant associations were observed in the fully adjusted models for attention or language domains, for either the MEDAS or MedDiet-GMS.

We further assessed the association between each of the 20 taxa comprising the MedDiet-GMS and trajectories of cognitive function. *Eggerthella*, which was negatively associated with higher adherence to MedDiet, was associated with a greater decline in executive function (FDR < 0.05) (Fig. 4 and Additional file2: Table S6). Similar associations were observed for *Enterococcus* in the language domain (FDR < 0.05) (Fig. 4 and Additional file2: Table S6).

Discussion

In this cohort of Spanish older adults with overweight or obesity and MetS, higher adherence to the MedDiet was associated with a gut microbial profile consistently linked to beneficial health outcomes and a more favorable cognitive trajectory over 6 years of follow-up. Participants with higher MedDiet adherence showed increased microbial diversity, distinct overall community composition, and enrichment of specific fiber- and polyphenol-metabolizing as well as SCFA-producing taxa. Importantly, we identified a novel gut microbial signature correlated with MedDiet adherence (MedDiet-GMS), which was also independently associated with more favorable long-term cognitive trajectories. To our knowledge, this is the first study to characterize a gut microbiota-based MedDiet adherence score that was prospectively associated with long-term trajectories of cognitive function. These findings support the hypothesis that, in older adults at risk of cognitive decline, both self-reported MedDiet adherence and its microbiome-derived counterpart reflect dietary patterns that may beneficially modulate the gut-brain axis and help mitigate cognitive decline during aging.

Consistent with previous studies, we observed that higher MedDiet adherence was associated with greater gut microbial richness and diversity [15, 54, 55], which are regarded as markers of a healthy gut ecosystem and have been linked to cardiometabolic and neurological health [7, 56, 57]. We also observed modest but significant differences in overall gut microbial composition

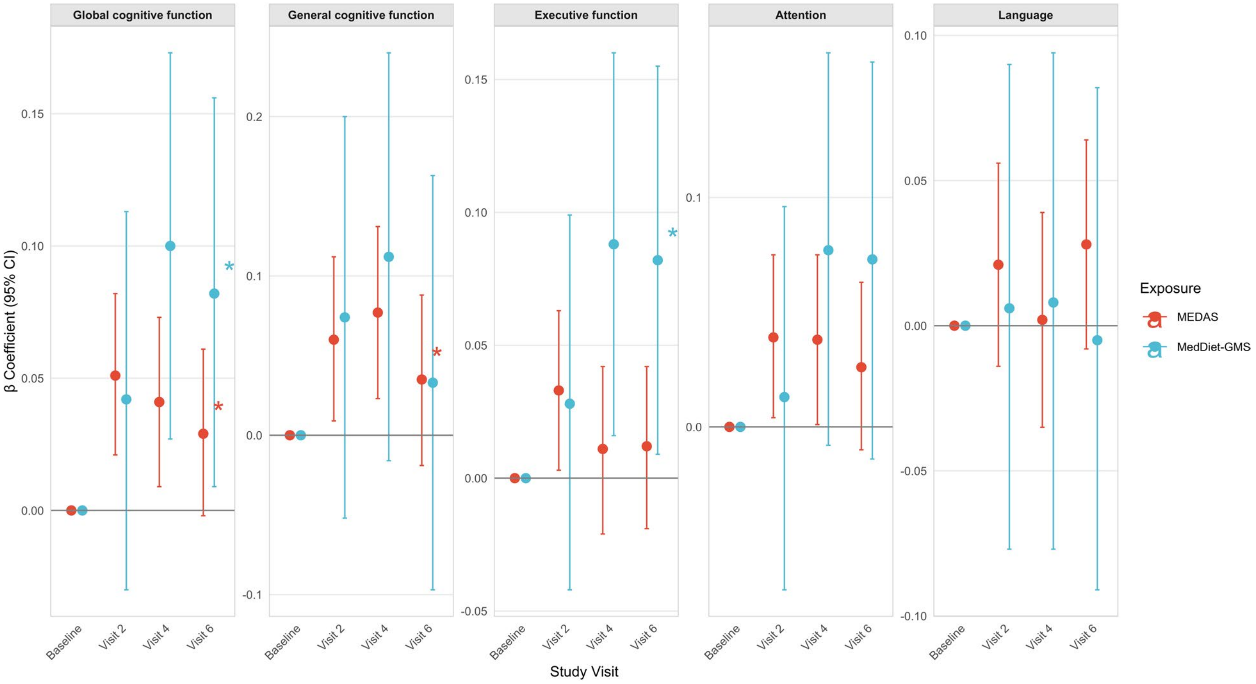

Fig. 3 Associations of MEDAS and MedDiet-GMS with cognitive function changes over follow-up time. β-coefficients represent the estimated difference in cognitive change (z-score) per unit increase in exposure at each follow-up visit compared to baseline, adjusted for covariates. Positive values indicate greater cognitive improvement (or less decline) associated with higher exposure values at that visit, while negative values suggest worse cognitive trajectories. Error bars show 95% CIs; intervals not crossing zero denote visit-specific significance ($p < 0.05$). *The significant overall interaction term ($p < 0.05$) indicates differential patterns of associations across all visits. Multivariable linear mixed-effect models included random intercepts for participants and adjusted for MEDAS or MedDiet-GMS, visit, and their interaction term (MEDAS*visit or MedDiet-GMS*visit); age (years); sex (male or female); recruiting center (Alicante, Barcelona, Reus, or Valencia); intervention group; educational level (primary, secondary, or college); marital status (single, divorced, separated, married, or widowed); baseline BMI (kg/m^2); baseline physical activity (METs-min/day); smoking status (never, former, or current smoker); baseline total energy intake (kcal/day); depressive symptomatology (yes or no); prevalence of diabetes (yes or no); hypertension (yes or no); and hypercholesterolemia (yes or no). Abbreviations: BMI, body mass index; CI, confidence interval; MEDAS, Mediterranean Diet Adherence Screener; MedDiet-GMS, Mediterranean diet related gut microbial score

according to MedDiet adherence levels. Among the individual components of the MEDAS, olive oil, vegetables and wine emerged as the primary contributors to variation in microbial composition in our study population. A common characteristic of these key MedDiet foods is their high content of polyphenols, a diverse class of bioactive phytochemicals with well-established antioxidant and prebiotic properties [14]. Due to their limited absorption in the upper gastrointestinal tract (approximately 5–10%), the majority of ingested dietary polyphenols are accumulated in the large intestine, where they are extensively metabolized by gut microbiota into phenolic metabolites with potential antioxidants, anti-inflammatory, and neuroprotective properties in maintaining gut barrier function, modulating immune responses, and promoting neuroprotection and cognitive health [7, 14]. In support of this, a previous cross-sectional analysis conducted within the same PREDIMED-Plus framework showed that the MedDiet was linked to higher levels of urinary phenolic metabolites derived from the gut microbiota, especially protocatechuic acid and enterolactone glucuronide, which were associated with better cognitive performance [58].

Our feature-level taxonomic analysis complements these earlier findings by identifying microbial genera associated with MedDiet adherence and its specific food components. In line with prior studies, we found that taxa such as *Barnesiella*, *Butyricicoccus*, *Agathobacter*, and *Bacteroides* were enriched in individuals with higher MedDiet adherence. These genera are well-known producers of short-chain fatty acids (SCFAs) [59], underscoring that a dietary pattern rich in plant-based foods may preferentially support and enrich bacteria that process dietary fibers and polyphenols into microbial bioactive metabolites. These metabolites play a pivotal role in maintaining gut mucosal integrity, regulating immune responses, and modulating brain health via the microbiota-gut-brain axis [7, 14]. Conversely, taxa such as *Acidaminococcus*, *Eggerthella*, and *Enterococcus*, which were negatively associated with MedDiet adherence in

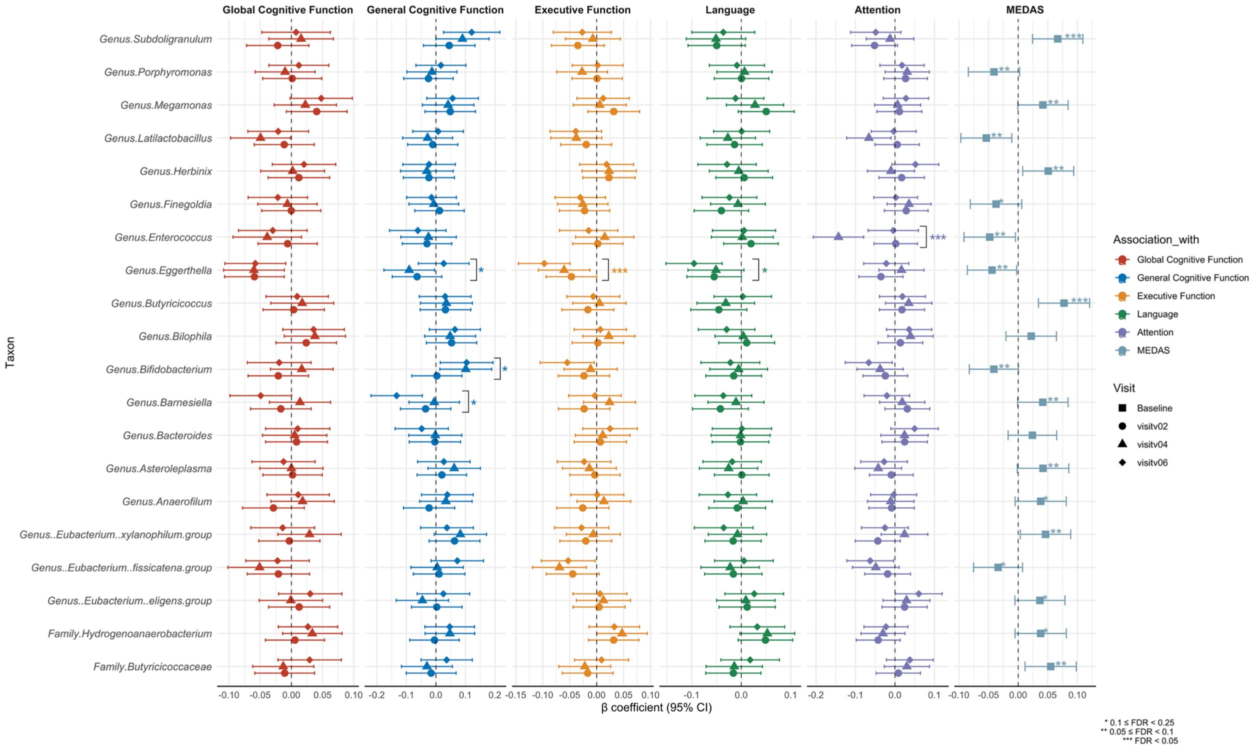

Fig. 4 Associations of the 20 taxa comprising the MedDiet-GMS with trajectories of cognitive function and baseline MedDiet adherence (MEDAS score). Values represent β coefficients (95% confidence intervals) derived from multivariable-adjusted linear regression models and linear mixed models. The linear regression models assessed associations between baseline MEDAS and log-transformed relative abundance of microbial genera constituting the MedDiet-GMS; adjusted for age (years); sex (male or female); recruiting center (Alicante, Barcelona, Reus, or Valencia); BMI (kg/m^2); physical activity (METs-min/day); smoking status (never, former, or current smoker); total energy intake (kcal/day); depressive symptomatology (yes or no); diabetes prevalence (yes or no); hypertension (yes or no); and hypercholesterolemia (yes or no). The linear mixed models evaluated associations between baseline MedDiet-GMS constituent genera and trajectories of cognitive function over time, adjusted for the same covariates plus group intervention. Statistical significance was determined using the Benjamini–Hochberg method to control the false discovery rate (FDR). For the linear regression models, FDR-adjusted *q* values reflect associations between MEDAS and taxa at baseline. For the linear mixed models, FDR-adjusted *q* values were derived from the interaction term (taxa × visit). Shapes denote different study visits, and significance levels are indicated as follows: *0.1 ≤ FDR < 0.25, **0.05 ≤ FDR < 0.1, ***FDR < 0.05. Abbreviations: MEDAS, Mediterranean Diet Adherence Screener; MedDiet-GMS, Mediterranean diet related gut microbial score

our study, have been previously linked to inflammatory states and adverse cardiometabolic, neurodegenerative, and psychiatric disorders [8, 57, 60]. These genera share a propensity for proteolytic metabolism and are often more abundant in western-style, low fiber, high saturated fat and animal-protein diets [7, 14, 61]. The observed composition shift, i.e., enrichment of SCFA-producing taxa and depletion of pro-inflammatory genera, aligns with findings from other large cohorts, including the Hispanic Community Health Study-Study of Latinos cohort and the Health Professionals Follow-up Study [53, 54], reinforcing the generalizability of the diet-microbiome interaction across diverse populations.

Extending previous evidence from the PREDIMED and PREDIMED-Plus trials, which reported cognitive benefits of the MedDiet [40, 62], in this PREDIMED-Plus sub-study, we leveraged gut microbiome data and employed a machine learning approach to derive a microbiome-based MedDiet adherence score (MedDiet-GMS). Importantly, we demonstrated for the first time that both self-reported MEDAS and MedDiet-GMS were independently associated with a more favorable trajectory in global cognitive function over 6 years. However, it should be noted that the MedDiet-GMS showed a modest correlation with MEDAS ($r = 0.38$) and limited predictive model performance ($R^2 = 0.15$, $Q^2 = 0.03$). The modest performance highlights the exploratory nature of the score and indicates that additional determinants beyond diet adherence are likely to shape microbiota-based signatures, underscoring that the MedDiet-GMS is not a direct substitute for MEDAS but rather a complementary microbiota-derived correlate. Interestingly, the associations of the MedDiet-GMS with cognitive trajectories were in some cases stronger than those of MEDAS, potentially reflecting the added contribution of these microbiota-related pathways that are relevant for cognitive aging. Notably, most of the microbial features contributing positively to the MedDiet-GMS (13 out of 20) were SCFA-producing taxa, further highlighting the potential role of SCFAs in supporting cognitive function. SCFAs can cross the blood–brain barrier and influence microglial activation, neurotransmitter synthesis, and brain-derived neurotrophic factor expression, all of which are mechanisms relevant to cognitive maintenance and neurodegeneration prevention [7, 14, 57]. Moreover, taxa that negatively contributed to the MedDiet-GMS, particularly *Eggerthella*, were found to be associated with a more pronounced cognitive decline in our study population, especially in the domain of executive function. *Eggerthella* has previously been associated with production of potential neurotoxic metabolites like p-cresol and phenylacetic acid, which may contribute to systemic inflammation disruption of blood–brain barrier (BBB) integrity, and neuronal dysfunction when elevated [63, 64]. This observation adds to the evidence that healthy dietary patterns like MedDiet may induce shifts in gut microbiota composition by enriching taxa and reducing the abundance of species associated with the production of pathogenic metabolites.

The higher risk profile of our study population, i.e., older adults with overweight/obesity and MetS, is important to consider when interpreting current findings. Both obesity and MetS have been consistently linked to an elevated risk of cognitive impairment and dementia, largely through mechanisms involving systemic inflammation, insulin resistance, and vascular dysfunction [1, 3, 17]. In parallel, these cardiometabolic conditions have been associated with perturbations in gut microbiota composition, including reduced microbial diversity and depletion of beneficial SCFAs–producing taxa, alongside enrichment of pro-inflammatory and metabolically detrimental species [65, 66]. Such microbiota alterations may exacerbate pathways that contribute to cognitive decline, including chronic low-grade inflammation, impaired BBB integrity, and altered neurotransmitter metabolism and signaling [67]. While this high-risk background underscores the potential relevance of dietary and microbiome-targeted strategies to mitigate cognitive decline in populations most vulnerable to dementia, it may also limit the generalizability of our findings to healthier populations. Future studies in cohorts with different demographic and clinical profiles will therefore be essential to confirm the broader applicability of these results.

There are practical challenges older adults may encounter when trying to adhere to healthy dietary patterns including the MedDiet, such as age-related conditions like social isolation, dentition problems, dysphagia, or gastrointestinal disorders. One strength of our study is the identification of a specific gut microbiome signature reflecting MedDiet adherence, offering a promising approach for translational applications and aiding adherence. Such microbial profiles may serve not only as objective biomarkers for dietary adherence, helping to complement or address for measurement error commonly observed in self-reported dietary assessments, but also as potential targets for microbiome-based therapeutics as a complementary or alternative strategy to preserve cognitive function and delay the onset of dementia. It is worth noting that although the effect sizes observed in our study were statistically significant, they were modest in magnitude. To contextualize their potential clinical relevance, we compared them to the impact of chronological aging. In our fully adjusted models, a 1-year increase in age was associated with a 0.051 decline in global cognitive z-scores (data not shown), whereas a 1-point higher MedDiet-GMS was linked to a 0.082 higher z-score unit of trajectory over 6 years. This suggests that, under consistent adherence, a 1-point MedDiet-GMS increase could roughly equivalent to offsetting 1.6 years of age-related cognitive decline. While this provides a useful benchmark for interpretation, it remains uncertain whether such effect sizes translate into clinically significant differences at the individual or population level. Further studies are needed to better define the clinical significance of the findings and to clarify the potential implications for cognitive health. Another notable strength of our study lies in the integration of dietary, microbiome, and cognitive data within the context of a well-characterized prospective cohort. Focusing on the MedDiet, a dietary pattern extensively validated for its various health benefits, allowed for a comprehensive assessment of dietary quality while minimizing the complexities and potential confounding inherent to studying individual nutrients or foods in isolation. Furthermore, the cognitive function outcomes were rigorously assessed using multiple validated cognitive tests and composite scores with both global and domain-specific cognitive trajectories over a substantial 6-year follow-up period evaluated, enhancing the robustness of the observed associations. Nonetheless, several limitations should be acknowledged. The MedDiet-GMS was developed using 16S rRNA gene sequencing at the genus level, which, despite its practicality for large-scale studies, lacks the taxonomic resolution required for species- or strain-level identification. In addition, the absence of direct measures of microbial metabolites, inflammatory markers, or BBB function limited our ability to validate mechanistic pathways and precluded robust cross-omics integration. While we observed enrichment of SCFA-producing taxa with higher MedDiet adherence, we were unable to quantify SCFAs or other microbially derived metabolites in plasma, making our biological interpretations partly inferential. Although a subsample of PREDIMED-Plus participants has plasma or fecal metabolomics and 16S rRNA data available, the overlap was too limited to enable adequately powered integrative analyses. Future studies incorporating larger samples with repeated, multi-omics assessments will be essential to confirm and extend these mechanistic insights. Additionally, the MedDiet shares many food components with other healthy dietary patterns, which could limit the specificity of the microbial signature to the MedDiet itself. Future investigations are warranted to explore whether microbial signatures can distinguish among different health-promoting dietary patterns, using metagenomic and metabolomic approaches. Another limitation is that gut microbiota was assessed only at baseline, without accounting for potential changes during the follow-up. Although the adult gut microbiota demonstrates a substantial degree of stability [68], it is also dynamic and can be modified by dietary changes, medication use, and health status over time [69]. This may introduce uncertainty when linking baseline microbiota profiles to long-term cognitive trajectories. However, it is worth noting that our microbiota analyses were aligned with baseline dietary assessments, reflecting overall lifestyle status at study entry, but precluding assessment of whether the derived MedDiet-GMS and its associations with MEDAS and other microbial features are maintained longitudinally. Future studies incorporating repeated microbiome measurements are needed to better capture these temporal dynamics and provide a more comprehensive understanding of diet-microbiota-cognition relationships. Large-scale prospective cohorts with repeated sampling can offer valuable insights into long-term associations, whereas RCTs with repeated microbiome and cognitive assessments would be particularly informative to strengthen causal inference and clarify mechanistic pathways. While the observational nature of this study limits causal inference, the prospective study design and application of linear mixed models help mitigate concerns regarding reverse causality, particularly for the microbiota–cognitive trajectory associations, since baseline microbiota profiles and diet-related microbial signatures were assessed prior to changes in cognitive outcomes. However, residual confounding cannot be entirely ruled out despite extensive adjustment for known covariates. Finally, the MedDiet-GMS was developed within a Spanish cohort of older adults with overweight/obesity and MetS. While this represents a clinically relevant high-risk population for dementia, it may also limit generalizability. Future studies in larger and more diverse cohorts are needed to determine whether this interplay of diet-microbiota-cognition differ according to different health status. Validation across diverse ethnic, racial, and geographical groups will be an important next step. Encouragingly, the direction and magnitude of our findings align with results from other cohorts, including studies conducted in Hispanic/Latino populations and the Health Professionals Follow-Up Study among US white male participants, suggesting broader relevance of diet-gut microbiome-cognition interrelations.

Conclusions

In conclusion, this study expands existing evidence by demonstrating that not only a self-reported MedDiet adherence but also a gut microbial signature correlated with MedDiet adherence were associated with favorable cognitive trajectories over time in older adults with overweight/obesity and MetS, thus, at higher risk of cognitive decline. Our results highlight the potential of microbiome-informed approaches both for monitoring dietary adherence and for developing microbiome-targeted strategies to support cognitive health in aging populations. Future research should prioritize the external validation of these microbial signatures across diverse populations and exploring their clinical utility in personalized nutrition and microbiome-based intervention for promoting healthy cognitive aging.

Abbreviations

ASVs	Amplicon sequence variants
BBB	Blood-brain barrier
BDI-II	Beck Depression Inventory
BMI	Body mass index
CDT	Clock Drawing Test
CLR	Centered log-ratio
DST	Digit Span Test
erMedDiet	Energy-reduced Mediterranean diet
FDR	False discovery rate
FFQ	Food Frequency Questionnaire
GCF	Global cognitive function
IQR	Interquartile range
LMM	Linear mixed-effects models
MEDAS	Mediterranean diet adherence screener
MedDiet-GMS	Mediterranean diet related gut microbial score
METs	Metabolic equivalents
MMSE	Mini Mental State Examination
PCoA	Principal coordinate analysis
PCR	Polymerase chain reaction
PERMANOVA	Permutational multivariate analysis of variance
PREDIMED-Plus	PREvención con DIeta MEDiterránea
RCTs	Randomized controlled trials
rRNA	Ribosomal RNA
SCFAs	Short-chain fatty acids
SSB	Sugar-sweetened beverages
TMT	Trail Making Tests
VFT	Verbal Fluency Tests
WAIS-III	Wechsler Adult Intelligence Scale-III

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12916-025-04488-y.

Additional file 1. Figures S1–S6. Fig S1—Flow chart of the study population. Fig S2—Directed acyclic graph for the interplay of adherence to the Mediterranean diet, gut microbiota, and trajectory of cognitive function. Fig S3—Results of gut microbial features robustly related with MEDAS, according to the elastic-net regression models for baseline MEDAS score. Fig S4—The correlation between MEDAS and MedDiet-GMS at baseline. FigS5—MedDiet-GMS and overall gut microbiota composition. FigS6—Associations of the MEDAS and its components and MedDiet-GMS with genus-level features. Tables S1–S5. Table S1—Composite cognitive function assessment equations. Table S2—Quantitative 14-item score of compliance with the Mediterranean diet. Table S3—17-item questionnaire to assess adherence to the energy-restricted Mediterranean diet, and its comparison with the original 14-item MEDAS. Table S4—Baseline distribution of the individual components of the MEDAS score. Table S5—Mixed-effect linear regression model for the associations of MEDAS and MedDiet-GMS with cognitive function changes over follow-up time.

Additional file 2. Table S6. Table S6—Associations of the 20 taxa comprising the MedDiet-GMS with trajectories of cognitive function and baseline MedDiet adherence (MEDAS score).

Acknowledgements

The authors thank all PREDIMED-Plus participants and investigators. CIBEROBN, CIBERESP, and CIBERDEM are initiative of the Instituto de Salud Carlos III (ISCIII), Madrid, Spain. The Hojiblanca (Lucena, Spain) and Patrimonio Comunal Olivarero (Madrid, Spain) food companies donated extra-virgin olive oil. The Almond Board of California (Modesto, CA), American Pistachio Growers (Fresno, CA), and Paramount Farms (Wonderful Company, LLC, Los Angeles, CA) donated nuts for the PREDIMED-Plus pilot study. The authors also thank the PREDIMED-Plus Biobank Network as a part of the National Biobank Platform of the ISCIII for storing and managing the PREDIMED-Plus biological samples.

Authors' contributions

All the principal PREDIMED-Plus investigators contributed to the study concept and design and to data extraction from the PREDIMED-Plus participants. JN, AHC, SKN, NB and JSS contributed to the concept and design of the present study. JN wrote the first draft, JN and AHC performed the statistical analyses under the supervision of SKN, NB and JSS. JN and JSS are the guarantors of this work and, as such, had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis. JN, AHC, SKN, NB, CB, PK, JV1, DC, OC1, JV2, IMI, LTC, OC2, MF, MRC, DDW, FJT, JSS substantively revised the manuscript for important intellectual content. All authors read and approved the final manuscript.

Authors' social media handles

X: @JiaQi_Ni (Jiaqi Ni), @JordiSalasSalva (Jordi Salas-Salvadó), @steph_nishi (Stephanie K. Nishi), @NanBabio (Nancy Babio).

Funding

This work was supported by the official Spanish Institutions for funding scientific biomedical research, CIBER Fisiopatología de la Obesidad y Nutrición (CIBEROBN) and Instituto de Salud Carlos III (ISCIII), through the Fondo de Investigación para la Salud (FIS), which is co-funded by the European Regional Development Fund (six coordinated FIS projects leaded by JSS and JVi, including the following projects: PI13/00233, PI13/00728, PI13/00462, PI14/01206, PI14/00696, PI16/00533, PI16/00366, PI16/00501, PI17/01441, PI17/00855, PI19/00017, PI19/00781, PI19/00576, PI20/00557, PI21/0046; the Especial Action Project entitled: Implementación y evaluación de una intervención intensiva sobre la actividad física Cohorte PREDIMED-Plus grant to JSS; the Recercaixa (number 2013ACUP00194) grant to JSS; grants from the Consejería de Salud de la Junta de Andalucía (PI0458/2013, PS0358/2016, PI0137/2018); the PROMETEO/2017/017 and PROMETEO/2021/21 grants from the Conselleria de Innovación, Universidades, Ciencia y Sociedad Digital from the Generalitat Valenciana; and by NIH grant R01DK127601. This research was also partially funded by the Eat2beNICE/H2020-SFS-2016-2 EU- H2020 European grant, and the Horizon 2020 PRIME study (Prevention and Remediation of Insulin Multimorbidity in Europe; grant agreement #847879). JN is supported by a predoctoral grant from Ministerio de Ciencia, Innovación y Universidades (FPU 20/00385). AHC is supported by a predoctoral grant from Martí Franquès – INVESTIGO research fellowship funded and supported by NextGenerationEU, Servicio Público de Empleo Estatal and Universitat Rovira i Virgili (2022PMF-INV-01). SKN is supported by a postdoctoral fellowship from the Canadian Institutes of Health Research (CIHR, MFE-171207). IMI was supported by the "Miguel Servet Type II" program (CPII21/00013) of the ISCIII-Madrid (Spain) and cofounded by the European Union. JSS, the senior author of this paper, was partially supported by ICREA under the ICREA Academia program. None of the funding sources took part in the design, collection, analysis, interpretation of the data, writing the report, or in the decision to submit the manuscript for publication.

Data availability

The datasets generated and analyzed during the current study are not publicly available due to data regulations and for ethical reasons, considering that this information might compromise research participants' consent because our participants only gave their consent for the use of their data by the original team of investigators. However, collaboration for data analyses can be requested by sending a letter to the PREDIMED-Plus steering Committee (predimed_plus_scom-mittee@googlegroups.com). The request will then be passed to all the members of the PREDIMED-Plus Steering Committee for deliberation.

Declarations

Ethics approval and consent to participate

All participants provided written informed consent. The trial protocol was reviewed and approved by the Comité d'Ètica d'Investigació Clínica (Reference ID: 13-07-25/7proj2) and by the research ethics committees of all participating institutions. The study was conducted in accordance with the Declaration of Helsinki.

Consent for publication

Not applicable.

Competing interests

JSS reports serving on the board of and receiving grant support through his institution from the International Nut and Dried Fruit Council, serving on the board of the Instituto Danone Spain and the International Danone institute. None of the other authors declare competing interests.

Author details

[1]Universitat Rovira i Virgili, Departament de Bioquímica i Biotecnologia, Alimentació, Nutrició, Desenvolupament i Salut Mental ANUT-DSM, C/Sant Llorenç, 43201 Reus, Spain. [2]Alimentació, Nutrició, Desenvolupament i Salut Mental, Institut d'Investigació Sanitària Pere Virgili (IISPV), Avda. Josep Laporte, 2, 43204 Reus, Spain. [3]Centro de Investigación Biomédica en Red Fisiopatología de La Obesidad y La Nutrición (CIBEROBN), Instituto de Salud Carlos III, Av. Monforte de Lemos, 3-5, 28029 Madrid, Spain. [4]School of Nutrition, Faculty of Community Services, Daphne Cockwell Health Sciences Complex, Toronto Metropolitan University, 288 Church St., Toronto, ON M5B 1Z5, Canada. [5]Clinical Nutrition and Risk Factor Modification Centre, St. Michael's Hospital, Unity Health 61 Queen St. E, Toronto, ON M5C 2T2, Canada. [6]Laboratory of Microbiology, Wageningen University & Research, Helix, Gebouw 124, Stippeneng 4, 6708, WE, Wageningen, The Netherlands. [7]Universidad Miguel Hernández, Instituto de Investigación Sanitaria y Biomédica de Alicante. (UMH. ISABIAL), Edif Gris, Avda Pintor Baeza, 12 HGUA, 03010 Alicante, Spain. [8]CIBER de Epidemiología y Salud Pública (CIBERESP), Instituto de Salud Carlos III, Av. Monforte de Lemos, Av. Monforte de Lemos, 3-5, 28029 Madrid, Spain. [9]Department of Preventive Medicine, University of Valencia, Av. Vicent Andrés Estellés, s/n, 46100, Burjassot, Valencia, Spain. [10]Unit of Cardiovascular Risk and Nutrition, Institut Hospital del Mar de Investigaciones Médicas Municipal d'Investigació Médica (IMIM), Dr. Aiguader, 88, 08003 Barcelona, Spain. [11]CIBER Diabetes y Enfermedades Metabólicas (CIBERDEM), Instituto de Salud Carlos III (ISCIII), Av. Monforte de Lemos, 3-5, 28029 Madrid, Spain. [12]Department of Endocrinology, Institut d'Investigacions Biomédiques August Pi Sunyer (IDIBAPS), Hospital Clinic, University of Barcelona, Carrer del Rosselló, 149,

Eixample, 08036 Barcelona, Spain. [13]Department of Endocrinology and Nutrition, Virgen de la Victoria University Hospital, the Biomedical Research Institute of Malaga and Platform in Nanomedicine (IBIMA-BIONAND Platform), University of Malaga, Avenida Severo Ochoa, 35, 29590 Malaga, Spain. [14]Department of Computer Languages and Systems, Jaume I University, Avinguda de Vicent Sos Baynat, S/N, 12006 Castelló de La Plana, Castellón, Spain. [15]Department of Preventive Medicine and Public Health, University of Navarra, Instituto de Investigación Sanitario de Navarra (IdiSNA), C/Irunlarrea, 3, 31008 Pamplona, Spain. [16]Channing Division of Network Medicine, Department of Medicine, Brigham and Women's Hospital and Harvard Medical School, 181 Longwood Avenue, Boston, MA 02115, USA. [17]Department of Nutrition, Harvard T.H. Chan School of Public Health, 655 Huntington Ave, Boston, MA 02115, USA. [18]Broad Institute of MIT and Harvard, Cambridge, Merkin Building, 415 Main St, Cambridge, MA 02142, USA.

Received: 6 June 2025 Accepted: 28 October 2025

References

1. Livingston G, Huntley J, Liu KY, et al. Dementia prevention, intervention, and care: 2024 report of the Lancet standing commission. Lancet. 2024;404:572–628.
2. Sachdev PS, Blacker D, Blazer DG, et al. Classifying neurocognitive disorders: the DSM-5 approach. Nat Rev Neurol. 2014;10(11):634–42.
3. World Health Organization. Risk reduction of cognitive decline and dementia: WHO guidelines. Geneva: World Health Organization; 2019. https://www.who.int/publications/i/item/risk-reduction-of-cognitive-decline-and-dementia. Accessed 23 Nov 2022.
4. Yassine HN, Samieri C, Livingston G, et al. Nutrition state of science and dementia prevention: recommendations of the Nutrition for Dementia Prevention Working Group. Lancet Healthy Longev. 2022;3:e501–12.
5. Fekete M, Varga P, Ungvari Z, et al. The role of the Mediterranean diet in reducing the risk of cognitive impairement, dementia, and Alzheimer's disease: a meta-analysis. Geroscience. 2025;47:3111–30.
6. Petersson SD, Philippou E. Mediterranean diet, cognitive function, and dementia: a systematic review of the evidence. Adv Nutr. 2016;7:889–904.
7. Schneider E, O'Riordan KJ, Clarke G, et al. Feeding gut microbes to nourish the brain: unravelling the diet–microbiota–gut–brain axis. Nat Metab. 2024;6(8):1454–78.
8. Loh JS, Mak WQ, Tan LKS, et al. Microbiota–gut–brain axis and its therapeutic applications in neurodegenerative diseases. Signal Transduct Target Ther. 2024;9(1):37.
9. David LA, Maurice CF, Carmody RN, et al. Diet rapidly and reproducibly alters the human gut microbiome. Nature. 2014;505:559–63.
10. Wu GD, Chen J, Hoffmann C, et al. Linking long-term dietary patterns with gut microbial enterotypes. Science. 1979;2011(334):105–8.
11. Rothschild D, Weissbrod O, Barkan E, et al. Environment dominates over host genetics in shaping human gut microbiota. Nature. 2018;555(7695):210–5.
12. Nagpal R, Neth BJ, Wang S, et al. Modified Mediterranean-ketogenic diet modulates gut microbiome and short-chain fatty acids in association with Alzheimer's disease markers in subjects with mild cognitive impairment. EBioMedicine. 2019;47:529–42.
13. Ghosh TS, Rampelli S, Jeffery IB, et al. Mediterranean diet intervention alters the gut microbiome in older people reducing frailty and improving health status: the NU-AGE 1-year dietary intervention across five European countries. Gut. 2020;69:1218–28.
14. Berding K, Vlckova K, Marx W, et al. Diet and the microbiota–gut–brain axis: sowing the seeds of good mental health. Adv Nutr. 2021;12:1239–85.
15. van Soest APM, Hermes GDA, Berendsen AAM. Associations between pro- and anti-inflammatory gastro-intestinal microbiota, diet, and cognitive functioning in Dutch healthy older adults: the NU-AGE study. Nutrients. 2020;12:3471.
16. Qureshi D, Allen NE, Kuźma E, et al. Metabolic syndrome and risk of incident all-cause dementia, Alzheimer's disease and vascular dementia: a systematic review and meta-analysis of longitudinal studies. Alzheimers Res Ther. 2025;17:1–13.
17. Senff J, Tack RWP, Mallick A, et al. Modifiable risk factors for stroke, dementia and late-life depression: a systematic review and DALY-weighted risk factors for a composite outcome. J Neurol Neurosurg Psychiatry. 2025. https://doi.org/10.1136/jnnp-2024-334925.
18. Alberti KGMM, Eckel RH, Grundy SM, et al. Harmonizing the metabolic syndrome. Circulation. 2009;120:1640–5.
19. Martínez-González MA, Buil-Cosiales P, Corella D, et al. Cohort profile: design and methods of the PREDIMED-Plus randomized trial. Int J Epidemiol. 2019;48:387–388o.
20. Salas-Salvadó J, Díaz-López A, Ruiz-Canela M, et al. Effect of a lifestyle intervention program with energy-restricted Mediterranean diet and exercise on weight loss and cardiovascular risk factors: one-year results of the PREDIMED-plus trial. Diabetes Care. 2019;42:777–88.
21. Sayón-Orea C, Razquin C, Bulló M, et al. Effect of a nutritional and behavioral intervention on energy-reduced mediterranean diet adherence among patients with metabolic syndrome: interim analysis of the PREDIMED-plus randomized clinical trial. JAMA. 2019;322:1486–99.
22. Folstein MF, Folstein SE, Mchugh PR. "Mini-mental state" a practical method for grading the cognitive state of patients for the clinician Related papers "Mini-mental state" a practical method for grading the cognitive state of patients for the clinician*. J Gsychiaf Res. 1975;12:189–98.
23. Blesa R, Pujol M, Aguilar M, et al. Clinical validity of the "mini-mental state" for Spanish speaking communities. Neuropsychologia. 2001;39:1150–7.
24. Shulman KI. Clock-drawing: is it the ideal cognitive screening test? Int J Geriatr Psychiatry. 2000;15:548–61.
25. Aprahamian I, Martinelli JE, Neri AL, et al. The clock drawing test: a review of its accuracy in screening for dementia. Dement Neuropsychol. 2009;3:74–80.
26. Del Ser Quijan T, García De Yébenes MJ, Sánchez Sánchez F, et al. Evaluación cognitiva del anciano. Datos normativos de una muestra poblacional española de más de 70 años. Med Clin (Barc). 2004;122:727–40.
27. Benton AL, Hamsher KSA. Multilingual Aphasia Examination. 3rd ed. San Antonio, TX: The Psychological Corporation; 1994.
28. Peña-Casanova J, Quiñones-Úbeda S, Gramunt-Fombuena N, et al. Spanish Multicenter Normative Studies (NEURONORMA Project): Norms for Verbal Fluency Tests. Arch Clin Neuropsychol. 2009;24:395–411.
29. Wechsler D. Wechsler Adult Intelligence Scale-III. San Antonio, Texas: The Psychological Corporation; 1997.
30. Wechsler D. WAIS III. Escala de inteligencia de Wechsler para adultos-III. Manual técnico. Madrid:TEA Ediciones 1999.
31. Peña-Casanova J, Quiñones-Úbeda S, Quintana-Aparicio M, et al. Spanish multicenter normative studies (NEURONORMA project): norms for verbal span, visuospatial span, letter and number sequencing, trail making test, and symbol digit modalities test. Arch Clin Neuropsychol. 2009;24:321–41.
32. Reitan R. Trail making test: manual for administration and scoring. Tuscon: Reitan Neuropsychology Laboratory; 1992.
33. Llinàs-Reglà J, Vilalta-Franch J, López-Pousa S, et al. The trail making test: association with other neuropsychological measures and normative values for adults aged 55 years and older from a Spanish-speaking population-based sample. Assessment. 2017;24:183–96.
34. Ni J, Nishi SK, Babio N, et al. Higher versus lower nut consumption and changes in cognitive performance over two years in a population at risk of cognitive decline: a cohort study. Am J Clin Nutr. 2023;118:360–8.
35. Fernández-Ballart JD, Piñol JL, Zazpe I, et al. Relative validity of a semi-quantitative food-frequency questionnaire in an elderly Mediterranean population of Spain. Br J Nutr. 2010;103:1808–16.
36. Moreiras O, Carbajal A, Cabrera L, et al. Tablas de composición de los alimentos. 9th ed. Madrid (España): Ediciones Pirámide; 2005.
37. Mataix VJ. Tablas de composición de alimentos. Instituto de Nutrición y Tecnología de Los Alimentos Granada (España): Editorial Universidad de Granada; 2003.
38. Schröder H, Fitó M, Estruch R, et al. A short screener is valid for assessing Mediterranean diet adherence among older Spanish men and women. J Nutr. 2011;141:1140–5.
39. Schröder H, Zomeño MD, Martínez-González MA, et al. Validity of the energy-restricted Mediterranean diet adherence screener. Clin Nutr. 2021;40:4971–9.
40. Nishi SKSK, Babio N, Gómez-Martínez C, et al. Mediterranean, DASH, and MIND dietary patterns and cognitive function: the 2-year longitudinal changes in an older Spanish cohort. Front Aging Neurosci. 2021;13:847.

41. Atzeni A, Martínez MÁ, Babio N, et al. Association between ultra-processed food consumption and gut microbiota in senior subjects with overweight/obesity and metabolic syndrome. Front Nutr. 2022. https://doi.org/10.3389/fnut.2022.976547.
42. Callahan BJ, McMurdie PJ, Rosen MJ, et al. DADA2: high-resolution sample inference from Illumina amplicon data. Nat Methods. 2016 13:7 2016;13:581–3.
43. Quast C, Pruesse E, Yilmaz P, et al. The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. Nucleic Acids Res. 2013. https://doi.org/10.1093/nar/gks1219.
44. Molina L, Sarmiento M, Peñafiel J, et al. Validation of the Regicor short physical activity questionnaire for the adult population. PLoS One. 2017;12:168148.
45. Beck AT, Steer RA, Brown GK. BDI-II. Beck depression inventory–II-Second Edition. Manual. San Antonio, TX: The Psychological Corporation; 1996. https://doi.org/10.1037/t00742-000.
46. Sanz J, Luis A, Carmelo P, et al. Adaptación española del Inventario para la Depresión de Beck-II (BDI-II): 2. Propiedades psicométricas en población general The spanish adaptation of Beck's Depression Inventory-II (BDI-II): 2. Psychometric properties in the general population. Clin Salud 2003;14:249–80.
47. A C. Estimating the population size for capture-recapture data with unequal catchability. Biometrics. 1987;43:783–91.
48. Shannon CE. A mathematical theory of communication. Bell Syst Tech J. 1948;27:379–423.
49. Simpson EH. Measurement of diversity. Nature. 1949;163:688. https://doi.org/10.1038/163688a0.
50. Aitchison J, Barceló-Vidal C, Martín-Fernández JA, et al. Logratio analysis and compositional distance. Math Geol. 2000;32:271–5.
51. Anderson MJ. A new method for non-parametric multivariate analysis of variance. Austral Ecol. 2001;26:32–46.
52. Mallick H, Rahnavard A, McIver LJ, et al. Multivariable association discovery in population-scale meta-omics studies. PLoS Comput Biol. 2021;17:e1009442.
53. Wang DD, Qi Q, Wang Z, et al. The gut microbiome modifies the association between a mediterranean diet and diabetes in USA Hispanic/ Latino population. J Clin Endocrinol Metab. 2022;107:e924–34.
54. Wang DD, Nguyen LH, Li Y, et al. The gut microbiome modulates the protective association between a Mediterranean diet and cardiometabolic disease risk. Nat Med. 2021;27:333–43.
55. Meslier V, Laiola M, Roager HM, et al. Mediterranean diet intervention in overweight and obese subjects lowers plasma cholesterol and causes changes in the gut microbiome and metabolome independently of energy intake. Gut. 2020;69:1258–68.
56. Joos R, Boucher K, Lavelle A, et al. Examining the healthy human microbiome concept. Nat Rev Microbiol. 2024 23:3 2024;23:192–205.
57. Valdes AM, Walter J, Segal E, et al. Role of the gut microbiota in nutrition and health. BMJ. 2018;361:36–44.
58. Domínguez-López I, Galkina P, Parilli-Moser I, et al. Microbial phenolic metabolites are associated with improved cognitive health. Mol Nutr Food Res. 2024. https://doi.org/10.1002/mnfr.202300183.
59. Fusco W, Lorenzo MB, Cintoni M, et al. Short-chain fatty-acid-producing bacteria: key components of the human gut microbiota. Nutrients. 2023;15:2211.
60. Nikolova VL, Hall MRB, Hall LJ, et al. Perturbations in gut microbiota composition in psychiatric disorders: a review and meta-analysis. JAMA Psychiat. 2021;78:1343–54.
61. Ross FC, Patangia D, Grimaud G, et al. The interplay between diet and the gut microbiome: implications for health and disease. Nat Rev Microbiol. 2024;22:671–86.
62. Valls-Pedret C, Sala-Vila A, Serra-Mir M, et al. Mediterranean diet and age-related cognitive decline: a randomized clinical trial. JAMA Intern Med. 2015;175:1094–103.
63. Wang X, Yang S, li S, et al. Aberrant gut microbiota alters host metabolome and impacts renal failure in humans and rodents. Gut. 2020;69:2131–42.
64. Shah SN, Knausenberger TB-A, Pontifex MG, et al. Cerebrovascular damage caused by the gut microbe/host co-metabolite p-cresol sulfate is prevented by blockade of the EGF receptor. Gut Microbes. 2024. https://doi.org/10.1080/19490976.2024.2431651.
65. Li R, Kurilshikov A, Yang S, et al. Association between gut microbiome profiles and host metabolic health across the life course: a population-based study. Lancet Reg Health. 2025;50:101195.
66. Holmes E, Li JV, Marchesi JR, et al. Gut microbiota composition and activity in relation to host metabolic phenotype and disease risk. Cell Metab. 2012;16:559–64.
67. Cryan JF, O'riordan KJ, Cowan CSM, et al. The microbiota-gut-brain axis. Physiol Rev. 2019;99:1877–2013.
68. Faith JJ, Guruge JL, Charbonneau M, et al. The long-term stability of the human gut microbiota. Science. 2013;341(6141):1237439.
69. Lozupone CA, Stombaugh JI, Gordon JI, et al. Diversity, stability and resilience of the human gut microbiota. Nature. 2012;489:220–30.

Publisher's Note