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SENIOR Protocol

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We use this protocol and it's working

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Abstract

Protocol to relate microbiota profiles with spontaneous brain activity in older people

Troubleshooting

Recruitment

1 The recruitment was performed through the GENYAL Clinical Trials Platform at IMDEA Food Institute. This study was approved by the institutional Research Ethics Committee (IMDEA Food Foundation) and performed in accordance with the principles of research involving human subjects stated in the Declaration of Helsinki (1964). All participants were clearly informed about the study methodology and provided written informed consent.

2 Inclusion criteria included: Age ≥ 55 years; BMI between 27–35 kg/m2; able to understand the informed consent; willing to comply with the study protocol. Exclusion criteria included: decreased cognitive function, pregnancy or breastfeeding, severe chronic health conditions (heart, liver, etc.), BMI >35 kg/m2, or pharmacological treatment for weight loss.

Anthropometric Measurements

3 Anthropometric measurements were collected following standardized methodology. Body Composition Monitor analyzer (BF511- OMRON HEALTHCARE UK, LT, Kyoto, Japan) was used to determine weight, % body fat, % skeletal muscle and visceral fat, as well as basal metabolic rate.

4 Height was measured with a stadiometer (Leicester-Biological Medical Technology SL, Barcelona).

5 Waist and hip circumference (cm) were measured using Seca 201 (Quirumed, Valencia, Spain).

6 Blood pressure and pulse were monitored with the Model M3 blood pressure monitor from OMRON HEALTHCARE UK, LT, Kyoto, Japan).

Behavioral Measurements

7 Sleep habits were assessed via the Oviedo Sleep Questionnaire (OSQ). The OSQ is a validated interview that support diagnosis of insomnia and hypersomnia during the previous month based on the DSM-IV and ED-10 criteria. The questionnaire is made up of a total of 15 items including Subjective satisfaction with sleep, Insomnia (difficulty with falling or remaining asleep, early awakenings or

restorative sleep, among others), hypersomnia (daytime sleepiness and its effects on daily tasks).

8 The psychological state of the participants was measured using the short version of the Depression, Anxiety and Stress Scale (DASS-21). designed to assess the emotional states of depression, anxiety, and stress. The DASS-21 consists of 21 items, divided into three subscales of 7 Likert items. A score for each scale is calculated by summing all the related items and these scores are later added to obtain a general score.

9 The Mini-Mental State Examination (MMSE) (Spanish version) is a widely utilized cognitive screening tool designed to assess cognitive impairment and dementia. The MMSE evaluate five cognitive domains:
orientation, registration, attention and calculation, recall, and language. The total score has a maximum punctuation of 35, with lower scores indicating greater cognitive impairment.

Fecal samples processing and 16S sequencing

10 Fecal samples were sent to Novogene for 16S sequencing of the bacterial V3V4 region: DNA was extracted by using Magnetic Soil and Stool DNA Kit (TianGen, China, Catalog #: DP712), following the manufacturer's protocol to ensure high-quality and high-yield DNA suitable for downstream applications.

11 The 16S rRNA gene was amplified using a set of universal primers targeting the V3-V4 regions of the 16S rRNA gene. The primers used were (5'- CCTAYGGGRBGCASCAG-3') and (5'-GGACTACNNGGGTATCTAAT-3'), which are known for their broad coverage of bacterial taxa while minimizing amplification of eukaryotic rRNA genes. The PCR conditions were optimized to ensure specific amplification, with an initial denaturation at 98°C for 1 minute, followed by 30 cycles of denaturation at 98°C for 10 seconds, annealing at 50°C for 30 seconds, and extension at 72°C for 30 seconds, with a final extension at 72°C for 5 minutes.

12 The amplified 16S rRNA gene fragments were performed by using specific primers connecting with barcodes. The PCR products of proper size were selected through 2% agarose gel electrophoresis. The same amount of PCR products from each sample was pooled, end-repaired, A-tailed, and further ligated with Illumina adapters. Libraries were sequenced on a paired-end Illumina platform to generate 250bp paired-end raw reads.

13 Raw sequencing data were processed using the DADA2 pipeline, which includes quality filtering, dereplication, chimera removal, and sequence variant inference. This method allows for the recovery of full-length 16S rRNA gene sequences with single-nucleotide resolution and a near-zero error rate, ensuring high accuracy in microbial community profiling.

14 Sequences were clustered into Operational Taxonomic Units (OTUs) using a de novo clustering approach, which has been shown to outperform reference-based methods in terms of stability and quality of OTU assignments. The clustering was performed using the VSEARCH algorithm, which is a viable open-source alternative to USEARCH.

15 Taxonomic classification of the OTUs was performed using the SILVA database, which provides comprehensive and up-to-date taxonomic information for 16S rRNA gene sequences. The classification was done at various taxonomic levels, including phylum, class, order, family, genus, and species.

EEG Data Acquisition

16 The EEG signal was acquired from 32 wet active ActiCAP electrodes (Brain Products GmbH, Gilching, Germany) placed on the scalp in the positions Fp1, Fp2, AF3, AF4, F7, F3, Fz, F4, F8, FC5, FC1, FC2, FC6, T7, C3, Cz, C4, T8, CP5, CP1, CP2, CP6, P7, P3, Pz, P4, P8, PO3, PO4, O1, Oz, O2 according to the 10-20 system. Reference was set to the Fz electrode and ground to the Fpz electrode. The signal was digitized and recorded by a actiCHamp amplifier (Brain Products GmbH, Gilching, Germany) at 256Hz. The impedance of all electrodes was kept under 10 kΩ.

17 The participants were comfortably seated with their forearms resting in their thighs, one meter away from a white wall.

18 They were asked to relax and breathe deeply with their mouth naturally open:

18.1 3 minutes eyes closed

18.2 One minute free resting

18.3 3 minutes eyes closed

EEG Processing

19 The EEG signal was preprocessed offline by a custom script in Matlab R2019b (The MathWorks Inc., Natick, MA, USA) using the EEGLAB (Delorme & Makeig, 2004) functions

19.1 Load channel locations.

19.2 Resample to 256Hz.

19.3 ASR with burst rejection (std > 20).

19.4 Bandpass filter (1Hz-31Hz).

19.5 ICA.

19.6 ICLabel artifact rejection.

19.7 PREP pipeline for only bad channel detection and interpolation

19.8 Rereference to average (CAR).

19.9 Channel interpolation by spectrum std > 1.5

20 Source density in six frequency bands (theta: 4Hz-7Hz; low alpha: 7Hz-10Hz; high alpha: 10Hz-13Hz; low beta: 13Hz-18Hz; mid beta: 18.5Hz-21Hz; high beta: 21Hz-30Hz) was calculated from the preprocessed signal by the eLORETA algorithm implemented in the LORETA-KEY software v20221229 .

21 Source density was averaged for each of the 210 cortical areas defined by the Brainnetome atlas.